H I L G A R D I A

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOL. 9 JANUARY, 1935 No. 2

COMPARATIVE HISTOLOGY OF HEALTHY AND PSOROSIS-AFFECTED TISSUES OF CITRUS SINENSIS[1, 2]

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INTRODUCTION

THE GENERAL CHARACTERISTICS and effects of psorosis (scaly bark) have been described by Fawcett[18] and Fawcett and Lee.[21] The first evident expression of the disease is the cracking or rupturing of the outer bark, with the formation of raised scales, pustules, or pimples. As the disease progresses, the wood underlying the affected bark is also commonly involved. The bark symptoms constitute the most conspicuous form of the disease and suggested the name psorosis originally given it by Swingle and Webber.[53] In ensuing years other effects were occasionally noted on small twigs, water-sprouts, mature leaves, and rarely on fruit. More recently a mosaic-like symptom on young leaves has been discovered by Fawcett,[20] suggesting a virus disease. Most of the histological work of this paper was completed before the mosaic-like symptom was discovered, and considerable attention was given to searching for the presence of possible microörganisms in the diseased tissues. This paper deals especially with the differences in structure of normal and diseased bark and wood. Some work in comparing normal and diseased twig and leaf tissues is also included.

MATERIAL AND METHODS

Specimens showing various stages of psorosis on Valencia orange trees nine years old were collected at different seasons of the year at the Citrus Experiment Station, Riverside, California. Through the

[1] Received for publication May 4, 1934.

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courtesy of J. C. Perry, specimens of bark and wood from trunks of Valencia orange trees seven years old, affected by psorosis, were collected monthly for an entire year at East Highlands, California. Additional specimens of diseased Valencia orange wood and bark were obtained in San Diego and Orange counties. Specimens from psorosis-affected Washington Navel orange trees were collected at East Highlands. As far as possible, healthy specimens approximating the diseased specimens in age were collected from groves in which the diseased trees occur.

Much of the material was sectioned and studied while fresh. The monthly collections from East Highlands were preserved in lactophenol. The remaining specimens were killed in formalin-acetic-alcohol or chrom-acetic-formalin. Stains generally employed to show the presence of fungi in host tissues were largely used. These included cotton-blue (Linder[32]), safranin (Moore[38]), Haidenhain's hematoxylin (Chamberlain[6]), gentian-violet, thionin (Stoughton[52]), and the combinations used by Cartwright,[5] Vaughan,[56] and Dickson.[12] Microchemical tests were made for fats and oils with Sudan III, for starch with IKI, and for gum with orcinol-HCl and phloroglucin-HCl, as outlined by Eckerson.[15] The chitosan reaction was employed in testing for chitin.

HEALTHY BARK

In this paper the term "bark" is used to designate those tissues outside the cambium. The literature pertaining to citrus bark as a whole is meager, the chief works being those of Moeller[37] and Penzig,[41] no reference to this genus being made by Mitlacher[35] in his discussion of rutaceous barks. There are, however, numerous references to special structures occurring within citrus bark, although many of these are made in connection with leaf anatomy. Mention of such works will be made as these particular structures are discussed.

The external appearance of the bark varies considerably as a stem increases in age. First-year shoots, which are angular at the apex and become rounded at the base, are smooth and glossy green throughout their length. On two-year-old shoots, slightly raised, vertically elongated lenticels begin to appear as light-gray streaks on a smooth, green background. In older stems, lenticels increase in number. At the age of five or six years, the bark is generally grayish or brownish with vertically elongated, slightly depressed green streaks. These green areas gradually decrease in number as the stem increases in age, until in time the bark is grayish or brownish over the entire stem. Throughout its life the bark remains smooth, except for very slightly raised, vertically elongated lenticels.

If a stem which has completed its first growing season is sectioned, the following primary permanent tissues included in bark may be observed to occur in centripetal order: epidermis, cortex merged with pericycle, and phloem (plate 1, figs. 1 and 2). In accordance with the observations of Moeller,[37] Penzig,[41] Douliot,[13] and Solereder[51] on *Citrus aurantium* and *C. limonum*, phellogen first appears immediately beneath the epidermis in stems several years of age. The phellogen produces phellem centrifugally and phelloderm centripetally; thus the periderm, which is persistent, is composed of these three layers. As has been previously pointed out by Moeller[37] and Damm,[10] the epidermis in *Citrus* commonly persists over at least a portion of the stem for many years (plate 3, fig. 1); hence, after the first two or three years the centripetal order of tissues comprising the bark is epidermis, periderm, primary cortex merged with pericycle, and phloem. The characteristics of these tissues in normal *Citrus sinensis* bark are described below.

Epidermis.—In surface view the ordinary epidermal cells are rather small, polygonal, with straight walls of variable thickness (plate 2, figs. 1–3). Over the internal oil glands the epidermis is commonly depressed and slightly modified. Here the cells are usually smaller, flatter, and concentrically disposed, as in the case of similar cells in citrus leaves figured by Penzig[41] and Tschirch and Oesterle.[54]

As may be seen from plate 2, figure 1, the epidermis is well provided with stomata. Although stomatal density is influenced considerably by age of the stem, for a given variety, the number per square millimeter on stems is probably always lower than that occurring on the lower side of the leaves (Reed and Hirano[46]). The guard cells are surrounded by accessory cells as in the case of stem stomata of *Citrus aurantium* figured by Penzig[41] (plate 2, figs. 1–2).

On first-year shoots, epidermal cells, with the exception of guard cells which contain chloroplasts, are mostly hyaline. However, toward the base of stems which have completed the first growing season and in the epidermis of older stems, cells with brown contents are to be found scattered singly or in small groups (plate 2). Frequently, the guard cells and accessory cells turn brown before the other epidermal cells.

Periderm.—Phellogen is first formed only in the layer of cortical cells immediately underlying groups of brown epidermal cells and brown cortical cells bordering the stomatal cavities (plate 2, figs. 4 and 5). The lateral extent of groups of phellogen cells in the early stages is frequently less than 12 cells. The cells are rectangular and radially flattened in transverse and radial sections, polygonal and approximately isodiametric in tangential section, and have dense granular protoplasm.

The phellogen soon gives rise to several layers of phellem, which

after a time rupture the epidermis by their growth. The cells of the phellem are radially arranged and readily traced back to the phellogen initials. At first they differ from the phellogen cells only in having greater radial diameters. Soon, however, small groups of cells in the phellem layers farthest from the phellogen become uniformly thick-walled (plate 2, fig. 5). Microchemical tests indicate that the thick walls of the phellem cells are lignified, while the thin-walled phellem cells are suberized. As the phellem increases in age, a notable increase in the relative abundance of lignified cells occurs until a lignified layer of greater radial depth than the suberized layer is produced. With continued production of phellem, alternating layers of lignified and suberized cells are produced (plate 1, figs. 3 and 4).

As the browning of the epidermis progresses, additional groups of phellogen cells are produced which give rise to phellem similar to that described above. In time, the isolated groups of phellogen are joined, and it becomes evident that phellem containing lignified cells is found only in lenticels. Because of the method of increase in lateral extent of the phellogen, several alternating layers of lignified and suberized cells are generally found near the center of a lenticel, while commonly but one lignified layer occurs at the borders of a lenticel. However, since the lignified layers of the lenticels are frequently attacked by various fungi (Peltier and Neal[40]), and subsequently by erosion, the number of these layers at the center of a lenticel may not greatly exceed that at the borders. The phellem produced between lenticels often develops without rupturing the epidermis. It consists solely of suberized cells similar to those found in the suberized layers of lenticels.

Phelloderm develops much more slowly than phellem. It is not visible in the early stages of phellem development but is found in limited amounts in stems possessing a phellogen which has been active for a considerable period of time (plate 3, fig. 1). The phelloderm cells are radially arranged and may be traced back to the phellogen layer without difficulty. They are parenchymatous, rectangular in transverse and radial sections, polygonal in tangential section, and contain chloroplasts.

Primary Cortex and Pericycle.—In current season's shoots the diameter of the primary cortex in transverse section is extremely variable. Near the apex where the stems are angular, the cortex is generally arched (plate 1, fig. 1). As the angular condition of the stem becomes lessened, the cortex loses its arched contour and forms a ring with approximately equal diameters (plate 1, fig. 2), a condition previously mentioned by Pitard.[44] Vesque[57] refers to the primary cortex as purely homogeneous. This term is not well chosen. Throughout its first year, the primary cortex generally is composed entirely of parenchyma,

but this shows considerable variation (plate 1, figs. 1 and 2), a fact referred to by Penzig.[41] Near the apex of such stems pericyclic fibers are scattered in small groups adjacent to the phloem. Near the base of first-year stems the pericyclic fibers often form a ring several cells wide (plate 1, figs. 1 and 2).

In the subepidermal layer of the primary cortex, crystal-bearing cells are developed in considerable numbers near the apex of first-year shoots. These apparently do not increase in number as the stem develops and hence become widely scattered as the stem increases in age. A fully developed crystal cell is considerably larger than other subepidermal cells, and further, differs from them in lacking chlorophyll and having thickened radial and inner walls to which a large, solitary calcium oxalate crystal surrounded by a membrane is attached (plate 2, fig. 2). With few exceptions, the long axes of subepidermal crystals are perpendicular to the epidermis. Crystal cells of this type were observed in citrus leaves by Payen[39] and Schacht.[47] Their developmental stages have been traced by Pfitzer,[42] von Guttenberg,[22] and Wittlin[60] in the lamina, and by Wakker[58] in the petioles of sour orange, *Citrus aurantium*, and by Kohl[29] in the petioles of sweet orange, *C. sinensis*, and grapefruit, *C. grandis*. The findings of these investigators are lacking in agreement.

With the exception of the crystal cells, the subepidermal layer is composed of thin-walled chlorenchyma which closely resembles the subjacent cortical tissue. The width of the chlorenchyma band is rather variable but generally exceeds six cells. Centripetally the cells become larger, thicker-walled, contain fewer chloroplasts, and show more pronounced tangential elongation. Between the chlorenchyma and pericyclic fibers, a band of colorless parenchyma tissue occurs (plate 1, figs. 1 and 2). At times some of the cells of this layer have irregularly thickened walls and probably should be referred to as weakly collenchymatous rather than parenchymatous. Many of the cells of this layer contain large, solitary calcium oxalate crystals which do not show definite orientation with respect to the periphery of the stem. In addition to calcium oxalate crystals, starch grains, oil droplets, and hesperidin (plate 7, fig. 4) were observed in primary cortical parenchyma cells.

In stems more than one year old, small irregular groups of stone cells begin to appear in the primary cortex, particularly in the colorless parenchyma zone (plate 1, fig. 3). Fully formed stone cells are characterized by much reduced lumina and thick, striated, lignified walls with numerous ramiform pits. In the development of stone cells from parenchyma cells, all cell dimensions, but especially the tangential diameters, are increased. Parenchyma cells bordering stone cells are accord-

ingly more or less crushed. In older stems, growth of the phelloderm and secondary phloem are also factors which doubtless result in partial or complete crushing of some of the cortical parenchyma cells.

Oil glands are formed very early in the development of the primary cortex (plate 1, figs. 1 and 2). They are close together near the stem apices but become rather widely separated in old stems. They commonly lie partly in the chlorenchyma and partly in the colorless parenchyma band. In form they are spherical or subspherical, their usual size in the material investigated being 90 to 210 μ. When fully formed a gland is commonly bordered by 2 to 6 concentric layers of epithelial cells with dense protoplasm and rich oil content. The interior of the gland is then characterized by fragments of cell walls and numerous oil droplets. Similar glands occurring in the leaves of various *Citrus* species have received the attention of numerous histologists. Such glands are regarded as lysigenous by Chatin,[7] von Höhnel,[27] Moeller,[37] DeBary,[11] and Penzig;[41] while Martinet,[33] Van Tieghem,[55] and Leblois[31] hold that they are schizogenous. The investigations of Sieck[49] on a number of rutaceous species including *Citrus aurantium* show that in reality the glands originate schizogenously and later become lysigenous.

Phloem.—As Moeller[37] and Penzig[41] point out, secondary phloem in *Citrus* is characteristically banded (plate 4, figs. 1 and 2). In first-year stems the phloem is composed of sieve tubes, companion cells, and phloem parenchyma. In older stems, bands of phloem fibers alternate with bands composed of the other phloem elements. The rings of both thick and thin-walled elements are broken by phloem rays.

Phloem fibers are closely crowded. Their lumina are nearly obliterated, while their walls are thick, lignified, and sparsely pitted. In transverse section they are chiefly angular and vary in diameter from 8 to 22 μ. They are generally about 400 to 675 μ long. The ends of fibers are rather abruptly tapered, usually serrate, and occasionally forked. The bands of fibers are commonly 20 to 100 μ in radial thickness.

Phloem parenchyma is abundant, both crystal-bearing and storage types occurring in fairly well-marked tangential bands in old as well as young phloem. The parenchyma cells of both types form vertical strands.

The crystal-bearing parenchyma borders the bands of phloem fibers and usually forms bands but one cell layer wide. Its cells are nearly cubical or somewhat vertically elongated, the usual diameter being 8 to 16 μ. Each cell contains a large, solitary calcium oxalate crystal. The cell walls are irregularly thickened.

Bands of storage parenchyma are usually one to four cells wide (plate 4, fig. 1). They occur adjacent to the bands of crystal-bearing paren-

chyma which border the bands of phloem fibers and also between such bands. The storage parenchyma cells are thin-walled and contain an abundance of starch grains and oil droplets. Usually they are 6 to 10 μ in radial diameter, 16 to 20 μ in tangential diameter, and 65 to 95 μ high.

The other bands of phloem tissue are composed of sieve tubes, companion cells, and scattered parenchyma cells of both types described above. In the band nearest the cambium, sieve-tube elements may be easily distinguished from parenchyma cells by means of their large central vacuoles, greater radial diameters, sieve plates, and accompanying companion cells (plate 4, fig. 1). The sieve tubes, companion cells, and scattered parenchyma cells in the sieve-tube bands soon undergo more or less radial crushing. Consequently in old phloem the lumina of all cells in such bands are practically obliterated, and protoplasts disappear, making it difficult to identify the various elements. Active sieve-tube elements are commonly 15 to 27 μ in tangential diameter, about 12 to 15 μ in radial diameter, and 110 to 160 μ long. Their end walls are horizontal or slightly oblique. Solitary sieve plates are confined to the end walls when these are horizontal, but five or more may occur in scalariform arrangement on the radial walls when the end walls are oblique. Companion cells are triangular or oblong in transverse section and vary greatly in size. A companion cell may lie across the entire radial wall of a sieve-tube element or it may border one corner of a sieve-tube element.

Phloem rays are homogeneous, uniseriate or multiseriate (plate 4, fig. 3). The uniseriate rays are 10 to 22 μ wide; 1 to 10 cells, 22 to 160 μ high. Multiseriate rays are 2 to 5 cells, 22 to 65 μ wide; and 4 to 27 cells, 55 to 375 μ high. The ray cells are chiefly radially elongated, oblong in transverse and radial sections, and circular in tangential section. In size they are generally about 11 to 19 μ in vertical and tangential diameters and 16 to 33 μ in radial diameter. The walls of the ray cells are thin and have numerous simple pits. Large calcium oxalate crystals, starch grains, and oil droplets were observed in the lumina of ray cells.

DISEASED BARK

External Appearance.—The earliest external symptom of psorosis on the bark is a roughening of the surface or exfoliating of small scales of outer bark over very limited areas of the trunk or large limbs. Such areas very gradually increase in size until the entire circumference of the trunk or limb is completely encircled. As the disease progresses the scales often become somewhat larger and involve the deeper layers of bark. Exudation of gum from affected areas may or may not accompany the scaling away of the bark.

Pathological Anatomy.—When bark showing the beginning stages of psorosis is sectioned, it exhibits differences from normal bark only in localized areas underlying the roughened surface. The striking histological feature of such bark is the occurrence of yellow to brown contents in the parenchyma cells of phelloderm and primary cortex underlying the affected surface (plate 5, figs. 1, 3, and 5). The cells with brown contents commonly occur in tangentially elongated groups (plate 5, fig. 3). Near the surface such groups may be rather extensive, but toward the interior of the primary cortex, they generally do not exceed three cells in the radial and vertical directions and two to four cells in the tangential direction, and frequently are only one cell wide and one cell high (plate 5, figs. 1 and 3). In some sections, isolated, brown cells are visible in the primary cortex. Brown contents may be observed in parenchyma cells which retain their normal form, but are more frequently met with in cells that are more or less crushed (plate 5, fig. 6).

Very soon after a group of brown cells is formed at the periphery, a subjacent phellogen layer extending to the normal phellogen is formed in the parenchyma of the primary cortex or phelloderm (plate 5, figs. 4 and 5). This produces phellem centrifugally and phelloderm centripetally. After a time the brown cells cut off by the corky layer are exfoliated in the form of a bark scale (plate 5, fig. 2).

The phellem is not unlike that which is normally produced. Generally it consists entirely of suberized cells (plate 3, fig. 2, and plate 5, fig. 2), but may be partly composed of lignified cells. However, phelloderm produced in bark affected by psorosis is commonly much more abundant than that normally formed in healthy bark. Moreover, it frequently contains in addition to parenchyma, broken tangential bands of stone cells (plate 3, figs. 2 and 3). These differ from the stone cells in healthy bark in that they are little, if any, larger than the parenchyma cells from which they are derived and are radially arranged within the groups.

As the disease progresses, additional groups of brown cells develop in the original phelloderm and cortex and in the newly formed phelloderm. These groups of brown parenchyma cells and any groups of stone cells which they may surround are soon cut off by subjacent periderm which develops as described above. After the tissues of the primary cortex are exfoliated, browning commonly continues to occur in the last-formed phelloderm layer. In severe cases it may extend to living portions of the phloem; when this occurs, subjacent phloem parenchyma subsequently gives rise to phellogen (plate 5, fig. 4).

Since browning of cell contents and subsequent periderm formation do not occur simultaneously throughout any given ring of cells, the phellogen ring becomes arched as the disease progresses (plate 5, fig. 4).

The arched contour of the phellogen, together with the abnormally large amount of phelloderm produced subjacent to brownish areas, doubtless creates abnormal pressure conditions in the underlying tissues. Hence, it is not surprising to find more or less distortion within such tissues.

In numerous parenchyma cells scattered from phelloderm to phloem, unusual contents were observed in one bark specimen collected in December and killed in chrom-acetic-formalin. A similar specimen collected from the same tree at the same time and killed in formalin-acetic-alcohol shows such contents to a far lesser extent. Such cell contents differ from the normal in including one to ten spherical, ovoid, or somewhat irregular-shaped masses varying in diameter from 2.5 to 11 μ. Such masses are deeply stained by thionin and gentian violet and become yellow when treated with potassium bichromate; they remain unstained, however, after treatment with Sudan III or phloroglucin and HCl. Because of the range of size which these bodies exhibit and the fact that each is apparently surrounded by a thin membrane, J. Dufrénoy has suggested that they represent vacuolar phenolic precipitates. It seems probable that this condition may represent an early stage in the development of brown-cell contents, for Dufrénoy[14] points out that a gentle excitation of any nature may be expected to induce the normally single vacuole of a healthy citrus cell to break into many smaller ones, while a more severe shock results in greater changes of vacuolar contents, which, in some cases, may be evinced by browning. A limited number of microchemical tests have indicated that the contents of brown cells in psorosis-affected tissues undergo a series of changes. In living material, such contents are frequently stained violet-red by neutral-red, and usually give a dark-green reaction with ferric chloride, indicating the presence of phenolic compounds. In some sections, Sudan III indicates the presence of some fatty substances. Very rarely, gum may be detected by means of phloroglucin-HCl or orcinol-HCl reactions. In killed material, the contents of brown cells usually show an affinity for stains which color lignin, but at times are impervious to stain.

In some instances, small gum pockets develop in the unusually thick phelloderm (plate 3, fig. 3). Such gum pockets involve only parenchyma tissue and evidently are schizolysigenous in origin. Contents of many of the cells bordering the cavity, as well as those of the cavity, give the reaction of gum upon treatment with phloroglucin-HCl or orcinol-HCl. Within some cells bordering the cavity or close to its margins, peculiar bodies stained by gentian violet are visible. Such bodies are chiefly spherical, about 4.75 to 9.50 μ in diameter, are bound by what appears to be a membrane about 0.5 to 1.2 μ thick, and commonly con-

tain a smaller globular structure. Generally, but one such body is present within a protoplast, but as many as three may occur.

In one badly diseased bark specimen from San Diego County, small groups of wood cells surrounded by cambium occur within phelloderm which has replaced the entire primary cortex and most of the phloem. These woody bodies resemble those described by Küster[30] in that they consist very largely of tracheids. However, occasionally they contain a few vessels.

HEALTHY WOOD[5]

Citrus wood has been described in more or less detail by Moeller,[36] Solereder,[50] Penzig,[41] Piccioli,[48] and Burgerstein.[2] From the descriptions of these authors, it may be concluded that structural differences between the woods of various species of *Citrus* are not well marked, since those that are pointed out are of a nature now known to be influenced by environment.

The *Citrus sinensis* wood studied is pale yellow, diffuse porous but with visible growth rings, hard, and of fine texture, the pores and rays being barely visible without a lens. With the aid of a lens the pores are seen to be fairly uniformly distributed throughout a growth ring, chiefly solitary or in radial multiples of 2 to 5, but occasionally in irregular or circular clusters of 3 to 5. Wood parenchyma is moderately abundant, chiefly paratracheal and metatracheal, but partly diffuse. Libriform wood fibers are the dominant element of the wood. Rays are commonly narrower than the pores, straight, or somewhat curved, and light colored, their distance apart being 1 to 3 pore widths. The minute structure of the various elements is described below.

Pores are chiefly circular to radially elongated; elliptic when solitary; and radially shortened, elliptic to angular when grouped. They vary in tangential diameter from 16 to 90 μ, the average diameter varying greatly in different specimens. The number of pores per square millimeter is also exceedingly variable in different specimens (plate 6, figs. 1 and 2), the observed range being 11 to 83. Vessel members are chiefly cylindrical, 34 to 285, mostly about 170 μ long. Perforations are simple, the plates horizontal, or slightly oblique. The lateral walls of vessels of secondary xylem are copiously pitted and lack spiral thickenings. Intervascular pit-pairs have roundish borders about 3 to 4 μ in diameter and included, slit-like apertures.

Metatracheal parenchyma bands are 2 to 6 cells wide. Both paratracheal and metatracheal parenchyma cells frequently contain stored

[5] Terms used in describing wood are those proposed by the Committee on Nomenclature, International Association of Wood Anatomists.[28]

food. They are about 10 to 15 μ in radial and tangential diameter, 60 to 135 μ high, and occur in strands. Their walls are thin and copiously pitted. Diffuse wood parenchyma strands are composed of 4 to 8 nearly cubical or somewhat vertically elongated cells about 22 to 43 μ high, each of which contains a large calcium oxalate crystal, which fills the lumen and is embedded in the irregularly thickened wall.

Libriform wood fibers are cylindrical in the central portion, tapering gradually, or sometimes abruptly at first, to smooth, saw-toothed, or rarely forked ends. Their usual diameter at the middle is about 10 to 16 μ, while their length varies from 250 to 900 μ, being chiefly about 500 μ. Their walls are about 4 μ thick with moderately numerous simple pits.

Both uniseriate and multiseriate rays are present (plate 6, fig. 10). The uniseriate rays are 7 to 11 μ wide, 1 to 13 cells (20 to 170 μ) high, and are homogeneous. Multiseriate rays are chiefly homogeneous, but occasionally slightly heterogeneous with upright cells restricted to the margins of the ray. They are 2 to 4 cells (17 to 45 μ) wide, 4 to 37 cells (54 to 435 μ) high, usually spindle-shaped, occasionally with uniseriate ends, rarely connected vertically. Both procumbent and upright cells have thin walls with numerous pits and commonly contain starch and oil. Procumbent cells are roundish to angular in tangential section, radially elongated, oblong in transverse and radial sections. Upright cells are conical to vertically elongated oblong in tangential section, squarish in transverse and radial sections.

DISEASED WOOD

Macroscopically, wood affected by psorosis differs from healthy wood in showing general discoloration or discolored concentric laminae from which gum may ooze when such wood is first cut. Often, affected wood is also gnarly.

Microscopically, the wood which is generally discolored is characterized by an abundance of gum in the vessel lumina. On transverse section, pores are often completely filled with gum (plate 6, fig. 6). Longitudinal sections show that, as a rule, this is due to the accumulation of gum in the vicinity of the perforation plates rather than to complete filling of the vessel lumina by gum.

Discolored laminae in the wood are due to the presence of traumatic gum ducts (plate 6, fig. 7). As in cases of gum-pocket formation in citrus wood described by Butler,[4] embryonic wood cells are involved. Accordingly, when present, gum ducts mark the beginning of successive growth rings, but they do not always completely encircle the stem. The distance between complete or partial rings of gum ducts is highly va-

riable, the observed range in one specimen being 27 to 1,350 μ. The gum ducts are apparently schizolysigenous in origin and vary considerably in size. Generally, they involve only cells between the rays (plate 6, fig. 11), extending vertically for considerable distances, tangentially from a few cells to the distance between rays, and radially from 30 to 165 μ.

Frequently the vertically elongated cells bordering the gum ducts are more or less completely filled with gum, but ray cells between gum ducts rarely contain gum. Occasionally, however, such cells contain abnormal globular or yeast-shaped masses (plate 6, fig. 8).

The tissues produced by the cambium subsequent to those involved in gum-duct formation are often normal in all respects. Not infrequently, however, some of the elements may become filled with gum, and at times patches of abnormal wood are formed. Such abnormal wood is homogeneous and lacking in elements of the form found in normal wood (plate 6, fig. 5). Its cells are nearly isodiametric, about 17 to 50 μ in diameter, with thin, lignified walls. In general aspect they resemble wood parenchyma, but the occurrence of weakly bordered pits in their walls indicates that they are of tracheid nature. They probably should be designated as parenchymatous tracheids, which, according to Küster,[30] are of frequent occurrence in wound wood. Some of these cells are completely filled with gum. Others contain globular or yeast-shaped bodies similar to those occasionally found in ray cells bordering gum ducts (plate 6, fig. 9).

In gnarly portions of the stem, all elements found in normal wood occur, but they assume somewhat altered forms and are arranged in different planes (plate 6, fig. 4). Such changes are doubtless correlated with changed planes of division in the cambium (plate 6, fig. 3), resulting from altered pressure conditions.

HEALTHY LEAVES

The leaves of *Citrus sinensis* are unifoliolate compound. Their structure is in general similar to that of citrus leaves described by Penzig,[41] Tschirch and Oesterle,[54] and Schulze.[48] The blade is bifacial, pinnately veined, glabrous, deep glossy green on the upper surface, and somewhat paler and duller on the lower surface. Oil glands appear as numerous translucent dots when the blade is held to the light. Hirano[26] gives the average area of full-grown leaves as 38.7 sq. cm in Valencia orange and 41.1 sq. cm in Washington Navel orange.

The upper epidermis is without stomata. Its cells are tabular with long axes parallel to the surface. The epidermal walls are straight and thick, the outer with a thick cuticle and the lateral with cutinized wedges

extending about half their depth. The cell contents are sparsely granular and hyaline. Over oil glands the epidermis commonly dips inward and the cells are concentrically arranged, often smaller and generally thinner-walled than the surrounding cells. According to the investigations of von Höhnel[27] and Haberlandt[24] on the leaves of sweet orange, *Citrus sinensis,* such modified structure of epidermal cells over oil glands permits emptying of the glands. Occasionally, crystal-bearing cells are found in the upper epidermis, but as von Guttenberg[22] has shown, these are of subepidermal origin and reach the epidermal layer through gliding growth. According to Butler[3] the upper epidermis often splits during development and is then replaced from the mesophyll.

The lower epidermis differs from the upper chiefly in that it possesses stomata, although the ordinary epidermal cells tend to be slightly larger, less angular, and more irregular in shape than those of the upper epidermis. Stomata are chiefly circular in surface view and are surrounded by accessory cells as in the case of citrus leaf stomata described by Schulze[48] and figured by Tschirch and Oesterle.[54] The usual diameter of stomata in the material studied is about 13 to 16 μ, but Bahgat[6] reports that stomatal size is influenced by environment. Stomata are less numerous in the vicinity of oil glands and large veins. Their density has been shown to be influenced by leaf size and age (Reed and Hirano[46]) as well as by environment (Bahgat). For mature *Citrus sinensis* leaves grown in full sunlight, Hirano[26] reports the average number of stomata per square millimeter in areas free from oil glands and large veins to vary from 402 ± 2.5 in the Lue Gim Gong orange to 533 ± 2.5 in the Ruby Blood orange, the numbers in Washington Navel orange and Valencia orange being respectively 458 ± 2.7 and 504 ± 2.7. In section, the stomata are seen to have prominent outer cuticular ridges and weakly developed inner cuticular ridges. Specific differences in the development of cuticular ridges of citrus stomata have been found by McLean.[34]

In very young *Citrus sinensis* leaves, the chlorenchyma shows little differentiation (plate 7, fig. 2), but in mature leaves, palisade and spongy parenchyma are distinct (plate 7, fig. 6). Halma[25] reports that leaves collected from the north and south sides of the tree, and those taken from upper and lower branches failed to show any differences in the degree of palisade development, but that leaves from the dense interior portion of the tree were markedly below the average in the de-

[6] Bahgat, M. M. A study of the structure and distribution of stomata in the different species of *Citrus.* Thesis for the degree of Master of Science, University of California, 1923. (Typewritten.) Copy on file in the University of California Library, Berkeley.

velopment of palisade tissue. He finds that the depth of palisade tissue is 23.8 ± 0.008 per cent of leaf thickness in Valencia orange, and 22.8 ± 0.12 per cent in Washington Navel orange. Usually, the palisade tissue consists of two rows of elongated cells, but at times a third row may be partially developed. The spongy parenchyma is rather dense. Its cells are fairly regular in shape; those adjoining the palisade tissue are generally circular in cross section, while those in the remaining layers are chiefly somewhat elongated parallel to the leaf surface.

Spherical or subspherical schizolysigenous oil glands (plate 7, fig. 2) similar to those found in the primary cortex are formed before palisade and spongy parenchyma are well differentiated. In mature leaves they occur in both types of chlorenchyma. They usually lie subjacent to or within a few cells of the epidermis. Cells containing solitary calcium oxalate crystals are very numerous (plate 7, figs. 1, 6, and 7). As in the case of stems, those occurring subjacent to the epidermis or pushed into the epidermal layer by gliding growth are characterized by strongly but irregularly thickened inner and radial walls which are attached to the crystal in fully developed leaves. Cells of this type are larger and somewhat more frequent in occurrence in the palisade than in the spongy parenchyma. In addition to occurring subepidermally, large calcium oxalate crystals are also scattered in the spongy parenchyma, particularly in the vicinity of large veins. Starch grains and hesperidin also occur in the mesophyll.

With the exception of the midrib, none of the veins project on the upper side of the leaf. On the lower side of the leaf, the midrib projects considerably and the major lateral veins slightly. Whether a correlation between size of vein islets and leaf maturity found by Ensign[16] in *Citrus grandis* also occurs in this species has not been determined. In section, two collateral vascular bundles are seen to pass through the central portion of the midrib (plate 7, fig. 1), while but one such vascular bundle is present at the apex of the midrib and in smaller veins. The lower vascular bundle of the midrib, which alone extends to the apex of the lamina, is crescent-shaped, and like those in smaller veins has dorsal phloem. The upper vascular bundle of the midrib has ventral phloem and toward the base of the lamina nearly closes the opening in the crescent-shaped lower bundle. At the base of the lamina the two collateral vascular bundles unite to form a single amphicribral vascular bundle. Rays are developed in the xylem portion of both upper and lower vascular bundles of the midrib. A narrow band of thin-walled fibers borders the phloem. Collenchyma extends from the band of fibers to the chlorenchyma, which is considerably reduced in the midrib.

DISEASED LEAVES

The first symptom of psorosis on leaves is the appearance of mosaic-like spots on rapidly growing young leaf blades. The mosaic effect is distributed over the entire lamina or only certain portions of it and is due to numerous elongated, light-colored areas 1 to 3 mm in length in the region of the smallest veinlets. The pale-green areas producing the mosaic effect commonly disappear or become masked as the leaf matures. Mature leaves may show few or many circular to irregular light-yellow to orange-colored areas varying in diameter from 0.5 to 10.0 mm. Frequently the discolored areas take the form of complete or partial narrow rings 2 to 7 mm in diameter. Raised, brown, corky areas are often present in the center of the yellow spots and also occur in the form of complete or partial rings of the same diameters as the yellow rings. Such corky areas occur on both upper and lower sides of the lamina and occasionally some of those on the two surfaces correspond in position.

Usually the tissues of mosaic-like areas of young leaves closely resemble those of healthy leaves of the same age except in chemical composition of cell contents as shown by staining reactions (plate 7, figs. 2 and 3). In leaves showing the mosaic effect, groups of epidermal and mesophyll cells with contents that stain heavily with safranin are far commoner than similar dark-staining cell groups in healthy leaves. In one diseased young leaf, a gum pocket was found in the mesophyll (plate 7, fig. 8).

When sections are made through the yellowish areas of mature leaves, ordinarily no departures from normal cell size, shape, or arrangement are evident. At this stage of the disease usually the only visible effect in the leaves is the yellowing or browning of contents of epidermal and, to some extent, of mesophyll cells in areas corresponding to the macroscopically discolored areas. In some instances, cells subjacent to those with darkened contents divide parallel to the leaf surface.

Sections through the corky areas of mature leaves show the presence of abnormal tissues at such points (plate 7, fig. 7). The cork on the upper surface of the leaf is produced by phellogen layers of limited extent produced in the palisade tissue, while that on the lower surface is formed by phellogen originating in the spongy parenchyma. Such phellogen layers may form in the mesophyll layers subjacent to the epidermis or at a depth of several cells in the spongy parenchyma but always originate subjacent to cells with brown contents. Towards the exterior the phellogen produces a phellem of tabular suberized cells, while towards the interior it forms a limited number of tabular parenchyma cells. At

about the time of periderm formation some of the cells underlying the periderm frequently divide. Such divisions are usually approximately parallel to the leaf surface. Scattered groups of brown cells are at times found in the mesophyll beneath the periderm. Not infrequently the leaf tissue external to the phellem is attacked by fungi of various kinds.

It is noteworthy that the histology of corky rings of *Citrus sinensis* leaves affected by psorosis corresponds closely to that of lesions attributable to other causes, indicating that as in cases cited by Butler,[3] similar reactions may result from different stimuli. Corky lesions of irregular form and unknown cause on orange leaves figured by Penzig[41] show similar arrangement of tissues in section. Penzig points out that browning of epidermal cells precedes the formation of such corky areas. Sections through lesions produced by *Sphaceloma fawcetti* Jenkins on citrus leaves are also similar (Cunningham,[9] Butler[3]). In citrus leaves wounded by mechanical means, Cunningham reports that a cicatrice is formed some distance from the edge of the wound, the intervening cells being dead and filled with a dense granular substance resembling tannin.

Although cork formation also occurs in citrus leaves from boron-deficient cultures, such leaves exhibit anatomical differences from leaves affected by psorosis. Haas and Klotz[23] have shown that in boron-deficient citrus leaves cork production is confined to the palisade tissue and veins.

DISCUSSION

A histological study of tissues affected by psorosis shows that the characteristics of the disease are: (1) the abnormal browning of contents of parenchyma cells, (2) abnormal periderm production, and (3) sometimes gum production. It is significant that both normal periderm formation in healthy stems and abnormal periderm formation in stems and leaves affected by psorosis occur subjacent to cells with contents that have turned brown. A similar restriction of periderm formation to tissues bordering browned cells has previously been reported in other genera (Küster,[30] Priestley and Woffenden[45]). Küster suggests that in such cases "some products of disintegration" or "unknown chemical combinations" produced in the brown cells incite cork formation in adjacent healthy tissue. Priestley and Woffenden consider that the essential antecedent to phellogen formation is the blocking of a parenchymatous surface, usually by a deposit of suberin or cutin which may occur in mixture with other substances around brown cells. Their experiments have shown that the development of phellogen amidst parenchyma follows the accumulation of sap at such a blocked surface.

Since abnormal browning of primary cortical parenchyma cells is the first visible symptom of psorosis in the bark of trunks, and a similar browning of epidermal and mesophyll cells is the first sign of psorosis in mature leaves, the determination of the nature of this browning is important in an understanding of the histology of the disease. The finding of mosaic-like symptoms and the transmission of the disease by budding and rooted cuttings suggest a virus as the possible initiating cause (Fawcett[19,20]). This suggestion is supported by the failure to find a microscopic causal organism, although the usual differential stains for fungi and the chitosan test were employed in examining affected tissue showing various stages of the disease, collected at different localities and at different times of the year. It is also supported by the striking similarity of some of the internal changes with those found in connection with many virus diseases; for example, the browning attended by hyperplasia in adjacent tissue is not unlike that accompanying definitely known virus effects.

Although the changes in the tissue bringing about the scaly condition of the bark are probably initiated by a virus, certain environmental conditions appear to influence these changes. As Fawcett[18] points out, in the majority of cases the apparent activity on the bark is quiescent during the winter and early spring and most pronounced during the summer and early fall.

That increased light intensity may be a factor accelerating the initial browning of cell contents in the cortex and leaves of citrus affected by psorosis is suggested by the fact that Cook[8] attributes localized browning in tomatoes to the pathological condition resulting from sunburn. That increased temperature, directly or indirectly through its influence on rate of transpiration, may be involved in the rate of browning of cells is suggested by the findings of Willison[59] and Priestley and Woffenden.[45] Willison[59] reports that browning of parenchymatous cells in wood and bark is influenced by temperature and moisture conditions. Priestley and Woffenden[45] indicate that the rate of blocking of a parenchymatous surface preceding cork formation is positively correlated with transpiration rate. As discussed above, in the case of citrus cortex and leaves, the browning of parenchymatous cells evidently represents such a surface blocking.

There is some evidence that factors increasing the transpiration rate may also be conducive to the formation of gum, which is often present in trees affected by psorosis. Fawcett[17] has shown that partial desiccation of tissues is not a necessary condition to the initiation of gum formation in citrus, but that it is one of the factors which accelerates gum formation in *Pythiacystis* gummosis. Bartholomew[1] indicates that

the water deficit in tissues resulting from excessive transpiration is also an important factor in gum production in endoxerosis of lemons. Since excessive transpiration is known to increase gum production in other diseases of *Citrus,* it is possible that it may be operative in accelerating gum formation in psorosis.

SUMMARY

In a healthy one-year-old stem of *Citrus sinensis,* epidermis, primary cortex and pericycle, phloem, cambium, xylem, and pith occur in centripetal order. Since the bark is persistent the order of tissues remains the same in later years except for the addition of periderm and loss of epidermis over parts of the stem.

Periderm formation commonly begins in the second year with the production of patches of phellogen subjacent to groups of epidermal cells with darkened contents. A continuous phellogen ring ordinarily does not form until several years later. Centripetally the phellogen produces a very small amount of parenchymatous phelloderm. Centrifugally it produces alternating layers of suberized and lignified cells in lenticels and suberized cells between lenticels.

In a psorosis-affected stem the first visible symptom of the disease is the abnormal darkening of contents of small, usually tangentially elongated groups of parenchyma cells of the primary cortex. This is followed by the production of a phellogen layer subjacent to the darkened cells. This phellogen produces phellem similar to healthy phellem centrifugally and phelloderm centripetally. The phelloderm is much more abundant than that produced by the phellogen of healthy stems, and at times contains radially arranged groups of small stone cells in addition to parenchyma similar to that of normal phelloderm. This abnormal production of tissue results in the formation of small, macroscopically visible eruptions on the bark surface. As the disease progresses, groups of parenchyma cells nearer the center of the stem become darkened and phellogen is formed subjacent to such groups. In time the tissues external to the abnormal phellem are sloughed off in scales which are the most conspicuous symptom of the disease on stems.

Gum which is often externally visible on psorosis-affected stems is commonly formed in the xylem and occasionally occurs in gum pockets formed in the abnormally thick phelloderm of such stems. Diseased xylem is characterized by concentric rings or partial rings of vertical gum ducts between the rays, and by scattered vessels partially plugged with gum.

In healthy, young leaves, oil glands and subepidermal crystal cells are differentiated before palisade and spongy parenchyma tissues be-

come distinct. In psorosis-affected leaves in which the mesophyll is not yet completely differentiated, a mosaic-like effect due to small light-colored areas in the lamina is macroscopically visible. Sections of such leaves differ from those of normal leaves in their staining reactions and are characterized by groups of epidermal and mesophyll cells which stain heavily with safranin. A gum pocket was observed in the mesophyll of one psorosis-affected leaf approaching maturity.

Mature leaves are distinctly bifacial, having stomata confined to the lower surface and a well-developed palisade layer commonly two or three cells in depth. In psorosis-affected, mature *Citrus sinensis* leaves, round to irregular discolored and corky areas, often in the form of complete or partial rings about 2 to 7 mm in diameter, are present in variable numbers. Sections through the discolored areas show an abnormal darkening of epidermal and occasionally mesophyll cells, but they usually otherwise closely resemble those of healthy mature leaves. At times, mesophyll cells subjacent to those with darkened contents may divide parallel to the leaf surface. Sections through corky areas of psorosis-affected mature leaves show that cork is produced by a phellogen formed subjacent to epidermal or mesophyll cells with darkened contents.

The apparent absence of a microscopic causal organism, together with the mosaic-like effect seen in young leaves and the browning of cells attended by hyperplasia in adjacent tissue, suggests that the disease may be caused by a virus. There is some evidence that its development may be accelerated by environmental factors.

LITERATURE CITED

[1] Bartholomew, E.T.
1928. Internal decline (endoxerosis) of lemons. VI. Gum formation in the lemon fruit and its twig. Amer. Jour. Bot. 15:548–563. Illus.

[2] Burgerstein, Alfred.
1908. Anatomische Untersuchungenen Samoanischer Hölzer. Denkschr. Math.-Naturw. Kl. Akad. Wiss. Wien. 84:466.

[3] Butler, E. J.
1930. Some aspects of the morbid anatomy of plants. Ann. Appl. Biol. 17:175–212. Illus.

[4] Butler, Ormond.
1911. A study on gummosis of *Prunus* and *Citrus* with observations on squamosis and exanthema of the *Citrus*. Ann. Bot. 25:107–153. Illus.

[5] Cartwright, K. St. G.
1929. A satisfactory method of staining fungal mycelium in wood sections. Ann. Bot. 43:412–413.

[6] Chamberlain, C. J.
1924. Methods in plant histology. 4th ed. 349 p. Illus. Univ. of Chicago Press, Chicago, Ill.

[7] Chatin, J.
1875. Études histologiques et histogeniques sur les glandes foliares interieures et quelques productions analogues. Ann. Sci. Nat. ser. 6 Bot. 2:199–221.

[8] Cook, M. T.
1921. Sunburn and tomato fruit rots. Phytopathology 11:379–380. Illus.

[9] Cunningham, H. S.
1928. Histology of the lesions produced by *Sphaceloma fawcetti* Jenkins on leaves of *Citrus*. Phytopathology 18:539–545. Illus.

[10] Damm, Otto.
1902. Ueber den Bau, die Entwicklungsgeschichte und die mechanischen Eigenschaften mehrjahrigen Epidermen bei den Dicotyledonen. Beihefte Bot. Centralbl. 7:219–260.

[11] DeBary, A.
1884. Comparative anatomy of the phanerogams and ferns. Clarendon Press, Oxford, Eng.

[12] Dickson, B.T.
1920. The differential staining of plant pathogen and host. Science n.s. 52:63–64.

[13] Douliot, H.
1889. Recherches sur le periderma. Ann. Sci. Nat. ser. 7, 10:325–395.

[14] Dufrénoy, J.
1929. A cytological study of water-soluble and fat-soluble constituents of *Citrus*. Jour. Agr. Research 38:411–429. Illus.

[15] Eckerson, S.H.
Microchemistry. (Mimeographed laboratory instruction notes.)

[16] Ensign, M. R.
1919. Venation and senescence of polyembryonic citrus plants. Amer. Jour. Bot. 6:311–329. Illus.

[17] Fawcett, H. S.
1923. Gummosis of *Citrus*. Jour. Agr. Research 24:191–236. Illus.

[18] Fawcett, H. S.
1931. Bark diseases of citrus trees in California. California Agr. Exp. Sta. Bul. 395:1–64.

[19] Fawcett, H. S.
1933. New information on psorosis or scaly bark of citrus. California Citrograph 18:326.

[20] Fawcett, H. S.
1934. Is psorosis a virus disease? Phytopathology 24:659–668.

[21] Fawcett, H. S., and H. A. Lee.
1926. Citrus diseases and their control. 582 p. 15 col. pl., 190 figs. McGraw-Hill Book Co., New York.

[22] Guttenberg, Hermann von.
1902. Zur Entwickelungsgeschichte der Krystallzellen im Blatte von *Citrus*. Sitz.-Ber. Math.-Naturw. Kl. Akad. Wiss. Wien. 111, Abt. 1:855–872. Illus.

[23] Haas, A. R. C., and L. J. Klotz.
1931. Some anatomical and physiological changes in *Citrus* produced by boron deficiency. Hilgardia 5(8):175–197. Illus.

[24] Haberlandt, G.
1898. Ueber den Entleerungsapparat der inneren Drüsen einiger Rutaceen. Sitz.-Ber. Wien. Akad. 107, Abt. 1:1221–1222. Illus.

[25] Halma, F. F.
1929. Quantitative differences in palisade tissue in citrus leaves. Bot. Gaz. 87: 319–324.

[26] Hirano, E.
1931. Relative abundance of stomata in *Citrus* and some related genera. Bot. Gaz. 92:296–310.

[27] Höhnel, F. R. von.
1881. Anatomische Untersuchungen ueber einige Sekretionsorgane der Pflanzen. Sitz.-Ber. Math.-Naturw. Kl. Akad. Wiss. Wien. 84, Abt. 1:576–577. Illus.

[28] International Assn. of Wood Anatomists, Committee on Nomenclature.
1933. Glossary of terms used in describing woods. Tropical Woods 36:1–12.

[29] Kohl, F. G.
1889. Anatomische physiologische Untersuchung der Kalksalze und Kieselsäure in den Pflanze. Marburo. *Reviewed in:* Guttenberg, Hermann von. Zur Entwickelungsgeschichte der Krystallzellen im Blatte von *Citrus*. Sitz.-Ber. Math.- Naturw. Kl. Akad. Wiss. Wien. 111, Abt. 1:855–872. 1902.

[30] Küster, Ernst.
1903. Pathologische Pflanzenanatomie. [Pathological plant anatomy.] 312 p. Illus. Gustav. Fischer, Jena. [Translation by Frances Dorrance. 258 p. 1913. (Mimeographed.)]

[31] Leblois, A.
1887. Canaux sécréteurs et poches sécrétrices. Ann. Sci. Nat. ser. 7, 6:265–270.

[32] Linder, David H.
1929. An ideal mounting medium for mycologists. Science 70:430.

[33] Martinet, J.
1872. Organes de sécrétions des végétaux. Ann. Sci. Nat. ser. 5 Bot., 14:91–232.

[34] McLean, F. T.
1921. A study of the structure of the stomata of two species of *Citrus* in relation to citrus canker. Bul. Torrey Bot. Club 48:101–106. Illus.

[35] Mitlacher, W.
1901. Vergleichende Anatomie einigen Rutaceen-Rinden. Zeitschrift des Allgemeinen Österreich Apotheker-Vereins. 39:225–232, 249–253, 278–280, 314–319. Illus.

[36] Moeller, J.
1876. Beitrage zur vergleichenden Anatomie des Holzes. Wien. Akad. Denkschr. 36, Abt. 2:297–426. Illus.

[37] Moeller, J.
1882. Anatomie der Baumrinden. Springer, Berlin. Illus.

[38] Moore, E. J.
1933. The use of phenosafranin for staining fungi on culture media or in host tissue. Sci. 77:23–24.

[39] Payen, M.
1846. Concretions et incrustations minerales. Mem. pres. par divers savants a l'Academie Sc. Math. et Phys. 9:91.

[40] Peltier, G. L., and D. C. Neal.
1918. Overwintering of the citrus canker organism in the bark tissue of hardy citrus hybrids. Jour. Agr. Research 14:523–524. Illus.

[41] Penzig, O.
1887. Studi botanici sugli agrumi e sulle piante affini. 590 p. and atlas of 58 pl. Tip. Botta, Rome.

[42] Pfitzer, E.
1872. Ueber die Einlagerung von Kalkoxalatkrystallen in die pflanzliche Zellhaut. Flora [Jena.] nr. 8:113–120; and 9:129–136.

[43] Piccioli, L.
1906. I caratteri anatomici per conoscere i principali legnami adopterati in Italia. Bullet. de Laborat. ed Orto Botan. Siena.

[44] Pitard.
1900. Nivellement et denivellement de la zone pericyclique heteromere dans les tiges agees. Actes Soc. Lin. Bordeaux 55:43. (Abstracted in: Just's Bot. Jahrsber. 29 part 2:403–404. 1901.)

[45] Priestley, J. H., and L. M. Woffenden.
1922. Physiological studies in plant anatomy. V. Causal factors in cork formation. New Phytologist 21:252–268.

[46] Reed, H. S., and E. Hirano.
1931. The density of stomata in citrus leaves. Jour Agr. Research 43:209–222.

[47] Schacht, H.
1845–6. Ueber die gestielten Traubenkorper. Abhdlg. Senckenberg Naturf. Gesellschaft 1:149–150.

[48] Schulze, Hilmar.
1902. Beitrage zur Blattanatomie der Rutaceen. Beihefte Bot. Centralbl. 12: 55–98. Illus.

[49] SIECK, W.
1895. Schizolysigene Sekretbehalter. Pringsh. Jahrb. 27:208–225. Illus.

[50] SOLEREDER, H.
1885. Ueber systematischer Werth der Holzstructur bei den Dicotyledonen. Munich.

[51] SOLEREDER, H.
1908. Systematic anatomy of the dicotyledons. (Translated by L. A. Boodle and F. E. Fritsch.) Clarendon Press, Oxford.

[52] STOUGHTON, R. H.
1930. Thionin and Orange G for differential staining of fungi and bacteria in plant tissue. Ann. Appl. Biol. 17:162. Illus.

[53] SWINGLE, W. T., AND H. J. WEBBER.
1896. The principal diseases of citrous fruits in Florida. U. S. Dept. Agr. Div. Veg. Phys. and Path. Bul. 8:1–42.

[54] TSCHIRCH, A., AND O. OESTERLE.
1895. Anatomischer Atlas der Pharmakognosie und Nahrungsmittelkunde. 351 p. Leipzig. Illus.

[55] VAN TIEGHEM, P.
1885. Canaux sécréteurs des plantes. Ann. Sci. Nat. ser. 7 Bot. 1:58–59.

[56] VAUGHAN, R. E.
1914. A method for the differential staining of fungous and host cells. Ann. Missouri Bot. Gard. 1:241–242.

[57] VESQUE, J.
1875. Memoire sur l'anatomie comparée de l'écorce. Ann. Sci. Nat. ser. 6 Bot. 2:82–198.

[58] WAKKER, J. H.
1888. Studien ueber die Inhaltskorper der Pflanzenzelle. Pringsh. Jahrb. 19: 423. Illus.

[59] WILLISON, R. S.
1932. Wound gum in peaches and grapes. Its relation to the invasion of fungus wound-parasites. Sci. Agr. 12:402–419, 484–505. Illus.

[60] WITTLIN, J.
1896. Ueber die Bildung von Kalkoxalat Taschen. Bot. Centralbl. 67:33–41, 65–73, 97–102, 129–133. Illus.

PLATES 1 TO 7

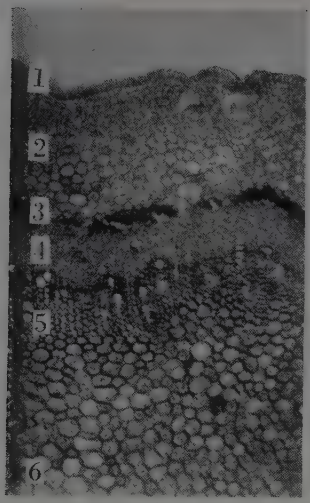
PLATE 1

Fig. 1.—Transverse section taken near the apex of a healthy one-year-old Valencia orange stem showing epidermis (*1*), primary cortex (*2*), pericyclic fibers (*3*), phloem (*4*), cambium and xylem (*5*), and pith (*6*). (×87.)

Fig. 2.—Transverse section taken near the base of a healthy one-year-old Valencia orange stem showing epidermis (*1*), primary cortex (*2*), pericyclic fibers (*3*), phloem (*4*), cambium and xylem (*5*). (×87.)

Fig. 3.—Transverse section of a healthy three-year-old Valencia orange stem showing periderm (*1*), stone cells in primary cortex (*2*), pericyclic fibers (*3*), and phloem fibers (*4*). (×87.)

Fig. 4.—Radial section of lenticel and subjacent cortex (*3*) of a healthy nine-year-old Valencia orange trunk showing narrow bands of suberized cells (*1*) alternating with bands of lignified cells (*2*). (×87.)



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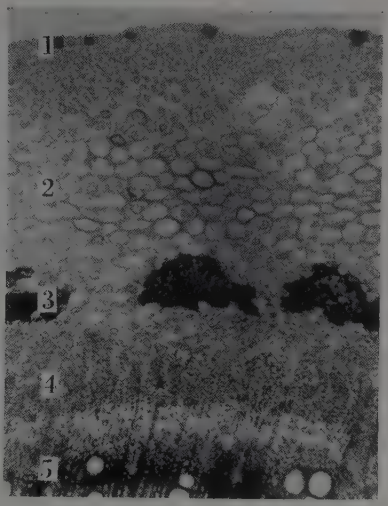

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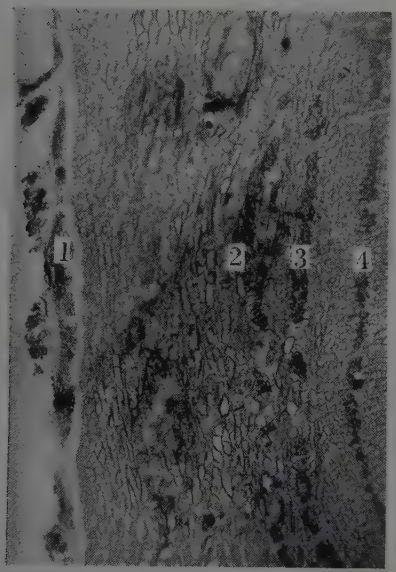

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PLATE 2

Key to Symbols: *1*, ordinary epidermal cell; *2*, stoma; *3*, subepidermal crystal cell; *4*, cortical parenchyma; *5*, suberized phellem; *6*, lignified phellem.

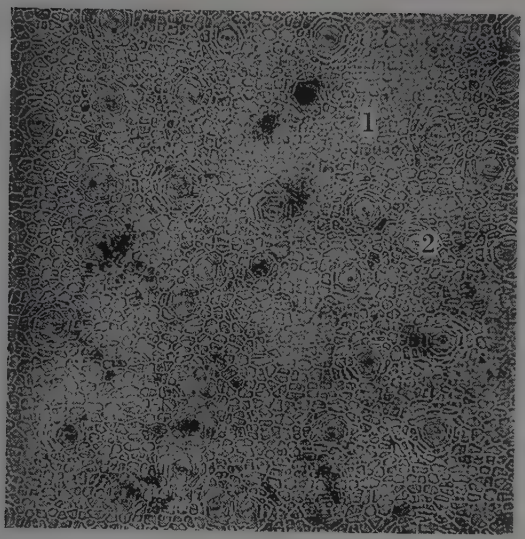
Fig. 1.—Surface view of epidermis from a healthy two-year-old Valencia orange stem showing distribution of ordinary epidermal and guard cells with darkened contents. (×120.)

Fig. 2.—Transverse section of epidermis and subjacent tissue of a healthy one-year-old Valencia orange stem showing subepidermal crystal cell and stomatal cavity. (×420.)

Fig. 3.—Transverse section of epidermis and subjacent tissue of a healthy two-year-old Valencia orange stem showing a group of cells with darkened contents. (×420.)

Fig. 4.—Transverse section of epidermis and subjacent tissue of a healthy two-year-old Valencia orange stem showing initial development of phellogen subjacent to a group of epidermal cells with darkened contents. (×420.)

Fig. 5.—Transverse section of epidermis and subjacent tissue of a healthy two-year-old Valencia orange showing a slightly more advanced stage of phellem development than in figure 4. (×420.)



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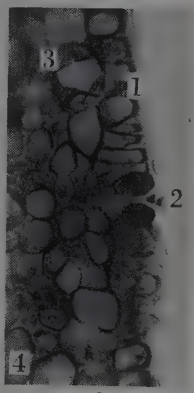

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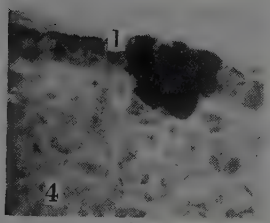

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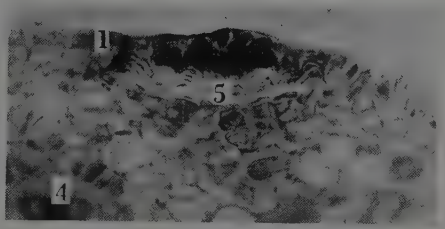

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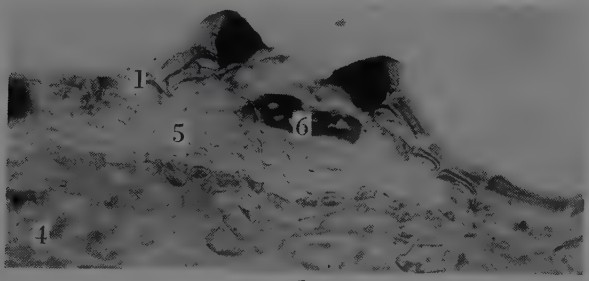


5

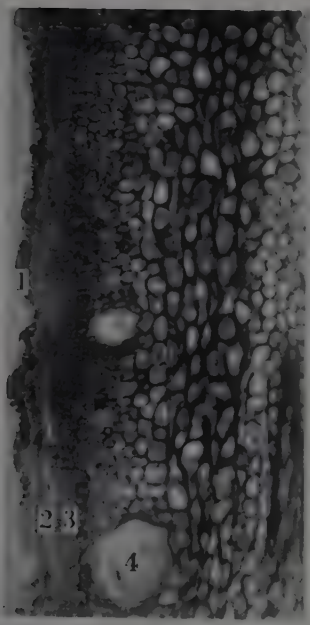
PLATE 3

Key to Symbols: *1*, epidermis; *2*, phellem; *3*, phelloderm; *4*, oil gland in the cortex; *5*, stone cells; *6*, gum pocket.

Fig. 1.—Radial section of outer bark of a healthy Valencia orange trunk showing persistent epidermis and periderm. (×120.)

Fig. 2.—Transverse section of abnormal periderm in psorosis lesion on trunk of a nine-year-old Valencia orange. (×120.)

Fig. 3.—Transverse section of a gum pocket in phelloderm of a psorosis lesion on the trunk of a nine-year-old Valencia orange tree. (×120.)



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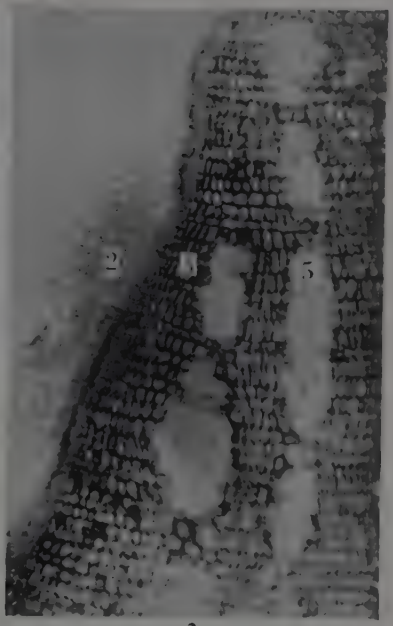

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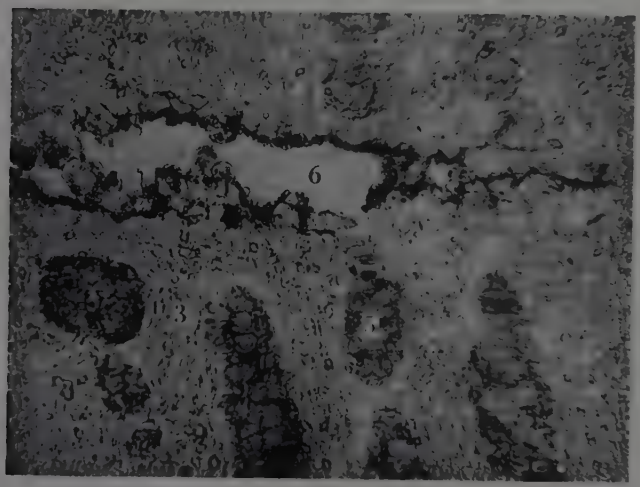

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PLATE 4

Key to Symbols: *1*, sieve tubes and companion cells; *2*, phloem parenchyma; *3*, phloem fibers; *4*, phloem ray.

Fig. 1.—Transverse section of the inner portion of the phloem in a healthy nine-year-old Valencia orange trunk showing occurrence of various tissues in tangential bands. (×120.)

Fig. 2.—Radial section of the inner phloem of a healthy nine-year-old Valencia orange trunk. (×120.)

Fig. 3.—Tangential section of active phloem of healthy nine-year-old Valencia orange trunk. (×120.)

1

2

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PLATE 5

Key to Symbols: *1*, tissue with abnormal brownish cell contents; *2*, abnormal periderm; *3*, stone cells; *4*, xylem.

Fig. 1.—Radial section of a young psorosis lesion on the trunk of a nine-year-old Valencia orange tree showing groups of parenchyma cells with darkened contents. (×90.)

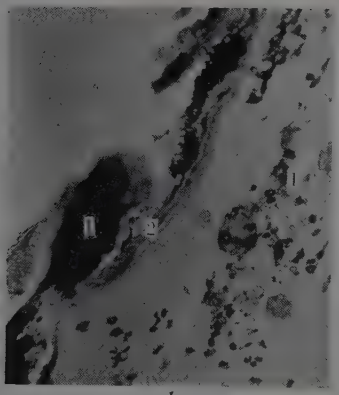
Fig. 2.—Transverse section of a young psorosis lesion on the trunk of a nine-year-old Valencia orange tree showing a portion of a small bark scale. (×90.)

Fig. 3.—Transverse section of a young psorosis lesion on the trunk of a nine-year-old Valencia orange tree showing tangentially elongated groups of parenchyma cells with darkened contents. (×90.)

Fig. 4.—Transverse section of a psorosis-affected Washington Navel orange twig showing abnormal periderm extending through the primary cortex to the xylem. (×90.)

Fig. 5.—Transverse section of a psorosis-affected Washington Navel orange twig showing abnormal periderm formed in the cortex and small groups of cortical parenchyma cells with darkened contents. (×90.)

Fig. 6.—Macerated cortical parenchyma cells with darkened contents from the trunk of a nine-year-old Valencia orange tree affected with psorosis. (×469.)



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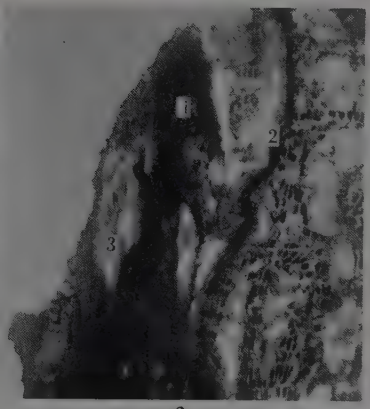

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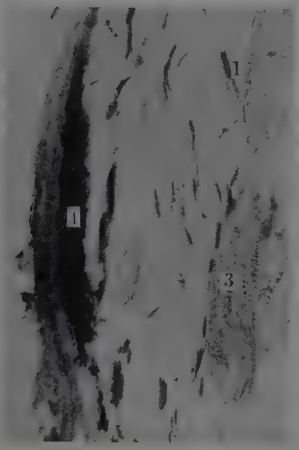

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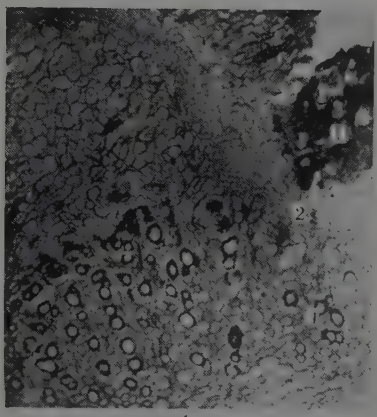

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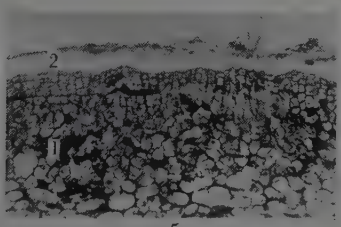

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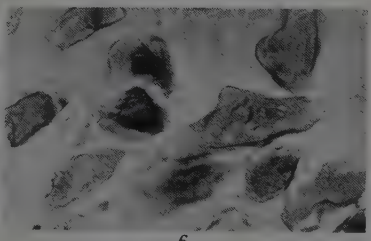

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PLATE 6

Key to Symbols: *1*, cambium; *2*, pore or vessel member; *3*, ray; *4*, gum duct; *5*, abnormal parenchymatous tracheid; all figures, Valencia orange.

Fig. 1.—Transverse section of cambium and subjacent xylem with few pores in a healthy trunk. (×80.)

Fig. 2.—Transverse section of cambium and subjacent xylem with numerous pores in a healthy large limb. (×80.)

Fig. 3.—Transverse section of cambium and subjacent xylem in a psorosis-affected branch showing irregular arrangement of tissues. (×80.)

Fig. 4.—Transverse section of xylem in a psorosis-affected branch showing irregular orientation of wood elements. (×80.)

Fig. 5.—Transverse section of xylem in a psorosis-affected branch showing a group of gum ducts and abnormal parenchymatous tracheids. (×80.)

Fig. 6.—Transverse section of discolored wood in a psorosis-affected branch showing gum in some of the vessels. (×80.)

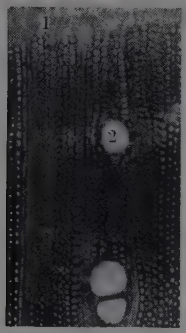
Fig. 7.—Transverse section of xylem in a psorosis-affected branch showing gum ducts. (×80.)

Fig. 8.—Abnormal cell contents in the xylem rays of a branch affected by psorosis. (×427.)

Fig. 9.—Parenchymatous tracheid in abnormal xylem produced subsequent to a gum pocket in a psorosis-affected branch. (×427.)

Fig. 10.—Tangential section of xylem in a healthy branch. (×80.)

Fig. 11.—Tangential section of xylem in the region of gum ducts in a psorosis-affected branch. (×80.)



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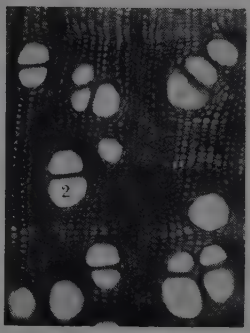

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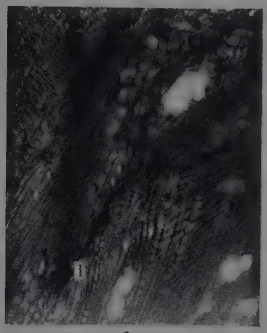

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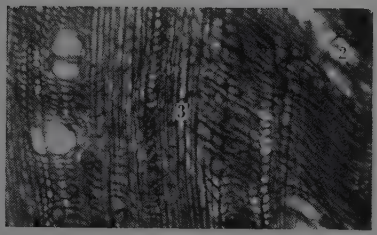

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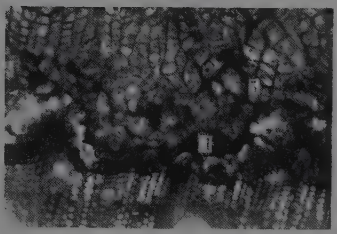

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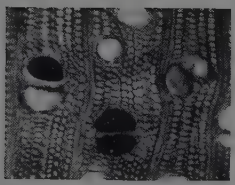

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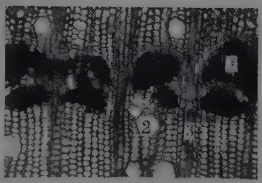

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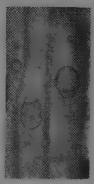

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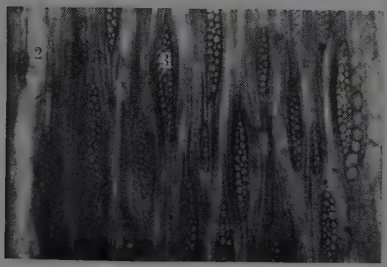

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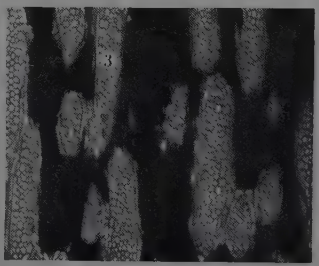

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PLATE 7

Key to Symbols: *1*, oil gland; *2*, subepidermal crystal cell; *3*, tissue with abnormal brownish cell contents; *4*, abnormal phellem; *5*, abnormal mesophyll cells.

Fig. 1.—Transverse section of the midrib taken at about the midpoint of a healthy mature Valencia orange leaf. (×90.)

Fig. 2.—Transverse section of a healthy young Valencia orange leaf. (×90.)

Fig. 3.—Transverse section of a psorosis-affected young Valencia orange leaf showing a group of mesophyll and epidermal cells with heavily stained contents. (×90.)

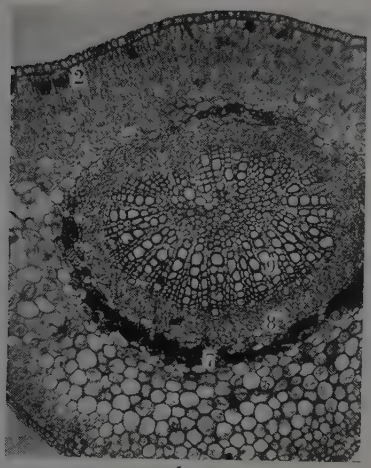
Fig. 4.—Hesperidin crystals in cortical parenchyma cells of a healthy one-year-old Valencia orange stem. (×469.)

Fig. 5.—Transverse section of a psorosis-affected mature Valencia orange leaf showing abnormal mesophyll subjacent to epidermal and palisade cells with darkened contents. (×90.)

Fig. 6.—Transverse section of a healthy mature Valencia orange leaf. (×90.)

Fig. 7.—Transverse section of a psorosis-affected mature Washington Navel orange leaf showing cork produced subjacent to epidermal cells with darkened contents. (×90.)

Fig. 8.—Transverse section of a psorosis-affected immature Valencia orange lamina showing a gum pocket in the mesophyll. (×90.)



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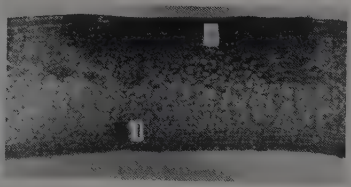

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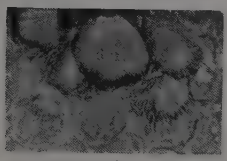

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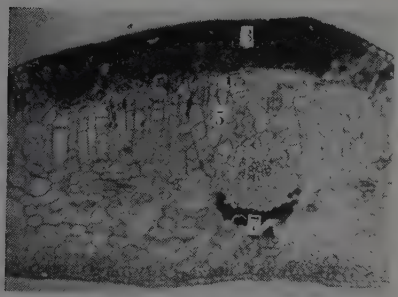

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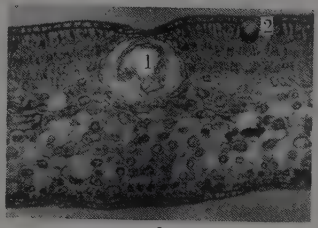

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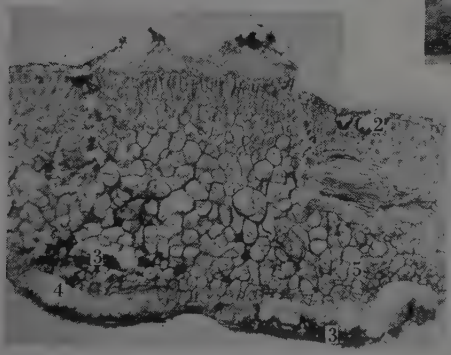

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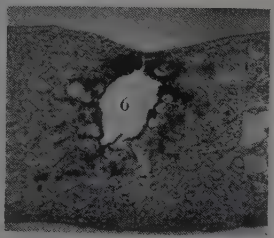

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THE EFFECTS OF ZINC AND IRON SALTS ON THE CELL STRUCTURE OF MOTTLED ORANGE LEAVES

H. S. REED AND J. DUFRÉNOY

THE EFFECTS OF ZINC AND IRON SALTS ON THE CELL STRUCTURE OF MOTTLED ORANGE LEAVES[1, 2]

H. S. REED[3] AND J. DUFRÉNOY[4]

INTRODUCTION

THE TERM "MOTTLE-LEAF" designates a functional disease of certain species and varieties of the genus *Citrus*. The most evident symptom of this disease is the absence of chlorophyll from certain areas between the veins of the leaf and, in more advanced stages, dwarfing of the leaves. The exact cause, or causes, of mottle-leaf have not been determined, but the evidence at hand supports the belief that the disease is independent of parasitic microörganisms. Climatic conditions often influence the occurrence and intensity of the disease, but it cannot be said that they have been shown to be the determining factor.

Soil conditions have been found to be more directly related to the occurrence of mottle-leaf. The application of nitrates (Vaile[20]) or of excessive amounts of urea (Haas[7]) may produce mottling of orange-tree foliage. The ratio of calcium to potassium also has certain pertinent relations to the disease (Kelley and Cummins,[10] Reed and Haas[17]), yet applications of calcium to the soil are by no means a corrective. Frequent applications of organic manures have been found to be one of the most satisfactory means in California for holding in check this disease, or for ameliorating the condition of trees badly affected with mottle-leaf.

The use of iron and zinc salts for the control of functional diseases of fruit trees has received much attention in California in recent years. Peach trees affected with little-leaf (Chandler, Hoagland, and Hibbard[5]) and orange trees affected with mottle-leaf (Johnston[9]) have shown striking benefits from applications of zinc sulfate singly or in combination with iron sulfate.

Mazé[12] pointed out a significant relation between zinc and sulfur metabolism in maize. He found that roots which were grown in a solution deficient in zinc were soon coated with an ocherous deposit. When traces

[1] Received for publication July 26, 1934.

[2] Paper No. 299, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[3] Professor of Plant Physiology in the Citrus Experiment Station and Graduate School of Tropical Agriculture and Plant Physiologist in the Experiment Station.

[4] International Fellow, Rockefeller Foundation.

of zinc were present, so that the plants could survive for a while, although suffering from inadequate supply of zinc, sulfur compounds were formed as a response. The presence of sulfides in the ash of the roots of the plants which received suboptimum amounts of zinc indicates that in them the process of sulfur metabolism was impaired. When a maize plant was transferred to a zinc-free solution, it died within a few days after the first symptoms of zinc starvation, and therefore had no time to react by the formation of sulfur compounds.

From these results Mazé concluded that zinc has a specific effect: in low concentrations it acts as a "food" for the cell, and its physiological action seems to be linked with sulfur translocation. However, zinc, when present in soluble form, is definitely toxic even at a very high dilution; for example, zinc nitrate at a concentration of 25 parts per million inhibited the growth of maize roots and killed the plants within one month, although under these conditions the amount of zinc was not sufficient to meet the zinc requirements of the plants.

Mazé[12] assumes that in the presence of calcium carbonate, zinc is precipitated as an insoluble salt from which necessary quantities are dissolved out by root excretions and absorbed as they are needed by the plant without any undue accumulation.

The importance of small quantities of zinc for the healthy growth of citrus has been shown by Haas[7] (pp. 488 and 489): "Where the cultures of citrus were maintained for periods of several years the addition of zinc was necessary to maintain health." And: " . . . . no other heavy metal in small concentrations has thus far been found to bring about the same beneficial response from the root system over a period of months or years."

On the other hand, concentrations of zinc higher than 5 p.p.m. may inhibit growth of citrus seedlings, as Haas[7] has shown.

Bertrand and Benzon[2] and Bertrand and Andreitcheva[1] found small quantities of zinc in the juicy part of citrus fruits (tangerines and oranges), but found larger amounts in chlorophyll-bearing organs. Their findings in relation to green and etiolated leaves are of particular interest in relation to the study of mottle-leaf. They showed that the green leaves of various salad plants were richer in zinc than etiolated leaves. It appears, therefore, that there may be a relation between zinc and chlorophyll production. Zinc may be assumed to play a rôle in chlorophyll production, or at least in some process in which chlorophyll is involved.

MATERIAL AND METHODS

The present article will be concerned mainly with the effects of zinc and iron salts on the cellular physiology of orange leaves, with reference to their beneficial effects on the mottle-leaf disease. The Division of Orchard Management of the Citrus Experiment Station has been studying the effects of applications of iron and zinc to the soil and has also sprayed certain badly mottled trees with a solution of zinc sulfate. Both of the treatments mentioned were followed by disappearance of the symptoms of mottle-leaf. Through the kindness of that division we had the opportunity of collecting material for cytological study.

The material designed for cytological study was fixed in Nemec's or in Meves' solutions. When prepared for micro-incineration, it was fixed in a mixture of equal parts of formalin and 95 per cent alcohol to avoid the introduction of chromates. The most favorable staining results were usually obtained by the use of hot acid fuchsin in aniline water, counterstained with toluidine blue and aurantia.

CYTOLOGY OF LEAVES

From an early stage the cells of mottled leaves are different from those of healthy leaves. The dissimilarity is indicative of deep-seated changes in the physiology of the affected leaves. The palisade cells of affected leaves are broad and often divided transversely, with the resulting formation of rhomboidal rather than columnar cells. When one examines tangential sections of leaves he finds that these large hypoplastic cells occur in rather definite groups with smaller cells of normal size intervening (Reed and Dufrénoy[15]).

The nuclei of these hypoplastic cells are elongated (fig. 3*B*) and have no nucleoli. Their appearance suggests a transformation from a sol to a gel condition.

The thin film of cytoplasm along the cell wall usually can be seen only by the use of the highest magnifications.

The strongest evidence of profound alterations in the physiology of the mottle-leaf cells is shown by their contents. The palisade and adjoining layers of cells are (as might be expected from the leaf color) very deficient in chloroplasts. The palisade cells generally have a polarized appearance due to the aggregation of cytoplasm and plastids at one end of the cell. Both plastids and mitochondria are in close contact with numerous small vacuoles of cytoplasm which show a honeycomb structure typical of hypoplastic cells. The chloroplasts of mottle-leaf cells generally contain thin, elongated starch grains which only partially fill the cavities in the plasts in which they lie. Probably the rates of hydroly-

sis of starch in such cells and translocation of sugars are greater than the rate of formation. A similar condition has also been observed in the cells of leaves affected with virus disease or in malnutrition. The stromata of the plastids are often rich in fat globules.

STARCH ACCUMULATION AND PHLOEM NECROSIS

The remarkable difference in the translocation of starch in green and mottled orange leaves has been mentioned in a previous paper (Reed and Dufrénoy[15]). Plastids showing fatty degeneration at one end frequently contain thin starch grains toward the other end. The fact that mottled leaves retain some starch after being kept in darkness for several weeks points to inhibition of starch utilization and starch translocation. This unhydrolyzed starch may indicate that amylase has not been produced, or that some by-product of the reaction has retarded its activity. The latter possibility seems to be in harmony with the necrotic condition of the phloem in the leaf veins.

The contents of the vacuoles of hypoplastic cells are rich in the class of phenolic material which is stained yellow by potassium bichromate or blackened by osmic acid. The former precipitates the phenolic substances either in the form of floccular precipitates or in the form of spherical granules, according to the degree of dispersion which existed in the vacuolar solution. One of the most important indexes of impaired physiological function is to be found in the occurrence of spheres of phytosterol or lecithin in the vacuoles. They probably represent material which the cell was unable to burn or to transform.

The fact that the emulsion of lecithin in water is capable of holding in solution or suspension a variety of substances which are otherwise insoluble, as, for instance, sterol, may explain how sterol, which is itself insoluble in water and can only be contained in the vacuolar watery solution in the form of colloidal indiffusible emulsion, might concentrate in the spherical inclusions here discussed. There are a number of ways in which the sterol might be conceived to alter the level of the autocatalyst of growth; for example, by modifying the action of lecithin or by exerting an effect of its own upon nuclear synthesis.

The lecithin-sterol inclusions in mottled leaves may be linked with the premature storage of starch and calcium in the leaf primordia in contrast to the deficiency of the same elements in adult leaves on mottled trees.

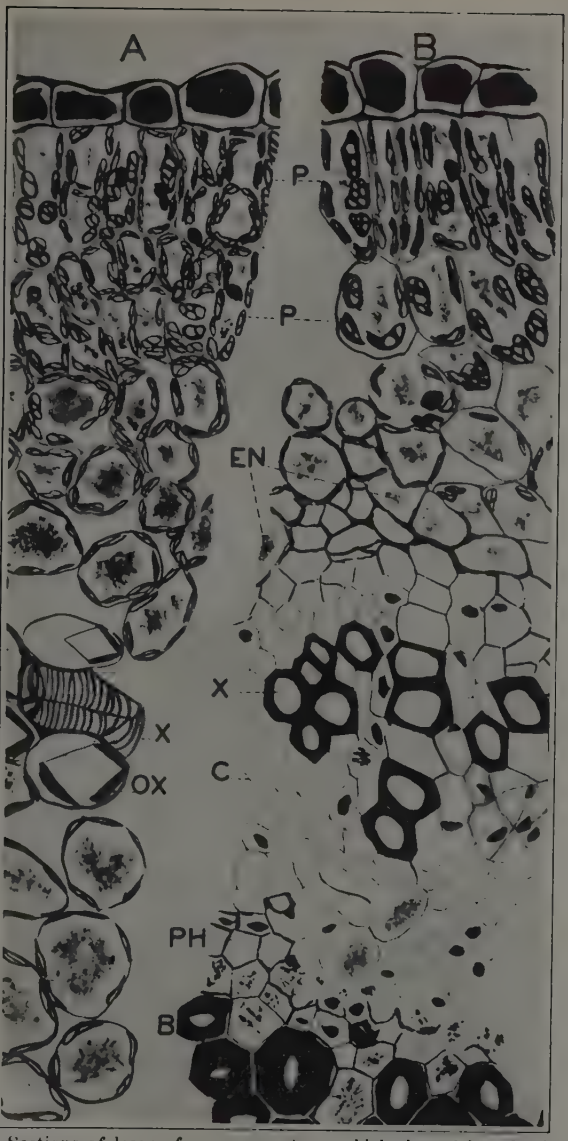


Fig. 1.—Sections of leaves from orange trees which showed beneficial effects of applications of 20 pounds of zinc sulfate per tree: *A* and *B* collected 15 and 17 months, respectively, after the applications were made. *P*, Palisade cells, many of which manifest the form which is characteristic of mottle-leaf palisade. However, they can have an abundance of healthy chloroplasts uniformly distributed in the cells. *B*, Bast fibers; *C*, cambium initials; *EN*, endodermis; *OX*, calcium oxalate; *PH*, phloem; *X*, xylem.

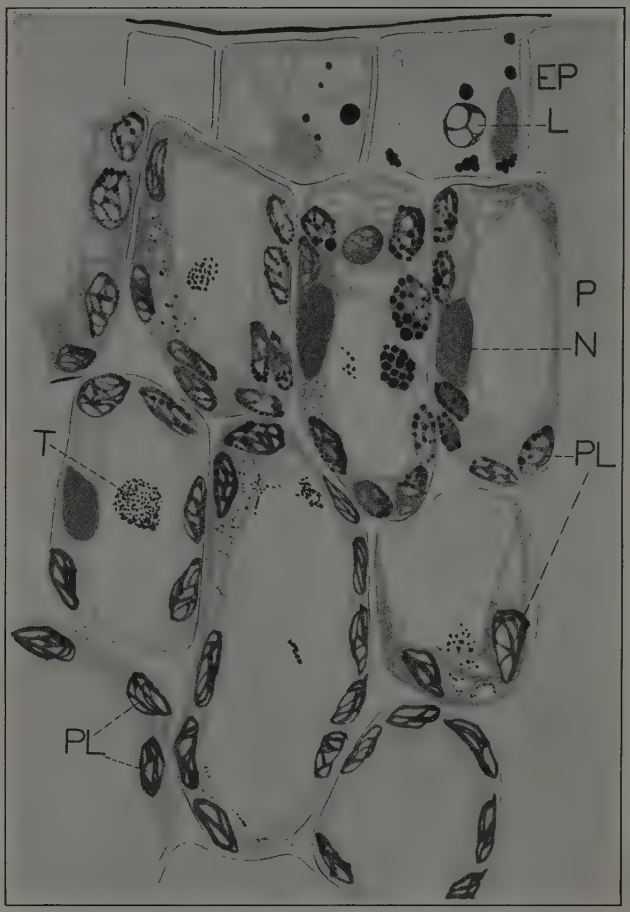


Fig. 2.—Section of orange leaf which showed beneficial effects of spraying with a 2.65 per cent solution of zinc sulfate. *EP,* Epidermis; *P,* palisade cells which retain the isodiametric dimensions characteristic of mottled leaves, although their contents show recovery from the hypoplastic condition; *T,* tannic substances; *PL,* plastids; *L,* lecithin.

THE EFFECTS OF ZINC ON THE CYTOLOGY OF LEAVES

Soil Treatments.—When one examines the cells of leaves from trees which were benefited by the application of zinc sulfate, one finds a different condition. The leaves show none of the irregular yellow areas and, naturally, the leaf cells contain healthy chloroplasts. The cell contents (fig. 1) are normally distributed in contrast to the characteristic clumping which prevails in the cells of mottled leaves.

The chloroplasts are regularly arranged in the peripheral regions of the cell, their stromata stain bright red with acid fuchsin, and they contain numerous starch grains. In the case of material killed with Meves' solution, tiny, dark oil drops show conspicuously in the bright-red background of the stromata (fig. 2).

Phenolic material, though present, is not abundant. The vacuolar inclusions composed of lecithin or phytosterol (which were present in the cells of mottled leaves) may be found in the cells of leaves from zinc-treated trees, but they are scarce.

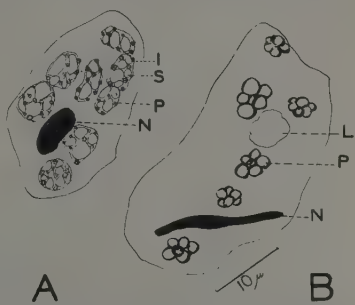


Fig. 3.—Cells from the mesophyll: *A*, normal condition in which chloroplasts, *P*, are regularly distributed, each of which contains numerous starch grains, *S*, and a few small oil droplets, *I*; *N*, nucleus. *B*, Hypoplastic condition from badly mottled leaf in which chloroplasts, *P*, contain many large oil droplets; *L*, lecithin; *N*, nucleus.

Many of the palisade cells of green leaves from zinc-treated trees are broad, resembling those of mottled leaves, and indicating that the tree may not have recovered fully (fig. 2). This condition is not surprising when the tremendous disorganization of cellular contents in badly mottled leaves is recalled. The succeeding cycles of leaves may show a closer approach to the normal organization of these complex cells. The appearance of leaf cells undergoing the changes incident to recovery are shown in figure 1. The trees from which the specimens were taken had received 20 pounds of zinc sulfate March 12, 1932. The drawings represent the cellular conditions 15 and 17 months later.

In mottled leaves, the chloroplasts contain many oil globules in the spring and early summer, as previously mentioned (Reed and Dufrénoy[16]). With the advance of the growing season, the number and size of the oil globules increase, eventually reaching a stage where the few small starch grains in the plastids may be masked by the surrounding large oil drops (fig. 3).

In contrast to that condition, the plastids of green leaves produced on trees in the Citrus Experiment Station grove which had been benefited by the previous application of zinc sulfate contained starch and a minimum of oil globules. Their stromata stained brightly, as did those in unaffected leaves, and showed normal starch grains, with very few small oil globules intervening.

Until now, we have made no specific study of the fibrovascular elements of the mottled or of the healthy orange leaf, although, as frequently noted, the tissue in the vicinity of the veins is the last to turn yellow when the leaf is affected with this disease. The cytological features of the fibrovascular bundles are described in the following paragraphs.

The xylem elements in affected leaves are interspersed with wood parenchyma, whose cells contain large vacuoles within which there is a large amount of phenolic material readily demonstrated with blue dyes or when the material is fixed with potassium bichromate or osmic acid (plates 1 and 2). The xylem elements in green leaves from orange trees which had been treated with zinc are more compactly grouped and may contain narrow medullary rays consisting of a row of cells whose cytoplasm is richly dotted with mitochondria, and whose vacuoles contain little phenolic material (plate 1). We have observed that these mitochondria in the xylem and phloem parenchyma frequently develop into plastids containing pigment, as shown in figure 5.

The phloem elements, even in healthy plants, often have a transitory existence, since they are superseded by, or crushed between, newly formed elements which differentiate from a cambial layer. A section of a vein from a green leaf from a tree which was treated with zinc shows that the phloem is mainly composed of living elements, each containing uniformly distributed mitochondria in the cytoplasm surrounding vacuoles wherein little, if any, phenolic material is demonstrable (plate 1).

The phloem elements in mottled leaves, on the contrary, show the following evidences of necrosis, even from an early stage: (1) overstaining of the nuclei and adjoining cytoplasm; (2) collapse of the cytoplasm accompanied by an aggregation of material at one end of the cell (polarization), presence of phenolic material in the vacuoles of many cells (plate 2) readily demonstrated when stained with blue dyes; (3) swelling of the pectic material in the middle lamella. The evidence of plasmolysis and of cytoplasmic disintegration may be seen in many cells, while those adjoining them retain a healthy appearance with abundant mitochondria in their cytoplasm.

The vascular bundles in petioles and leaves are surrounded by the

pericycle layer (plates 1 and 2). In the vicinity of the xylem elements, the pericycle consists of a row of cells containing large vacuoles in which phenolic compounds are abundant. The contents of these vacuoles,

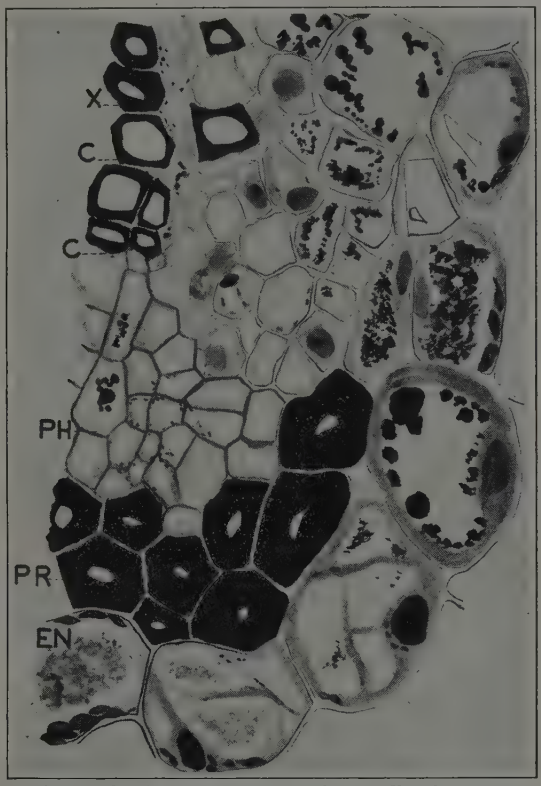


Fig. 4.—Section of a small vascular bundle of an orange leaf from a tree which had been treated 17 months earlier with zinc sulfate. Leaves had a healthy green color. *EN*, Endodermis; *PE*, pericycle; *X*, xylem; *PH*, phloem; *C*, cambium.

in green as well as in affected leaves, are readily precipitated when penetrated by vital dyes or by killing fluids containing chromates. The phenolic materials are precipitated in the form of small aggregates. The pericycle in contact with the phloem in green leaves forms a compact strand of fibers (plate 1). The pericycle fibers in mottled leaves show the same tendency to disperse which we have already noted in the xylem (plate 2).

The endodermal layer (plates 1 and 2) consists of cells which alternate with those of the pericycle, forming a sheath (the so-called "starch sheath") around the fibrovascular bundle. In the orange leaf we have found, however, relatively few amyloplasts in endodermal cells, and they are crowded closely to the outer wall of the cell through the expansion of the large central vacuole (fig. 4). The phenolic material in the

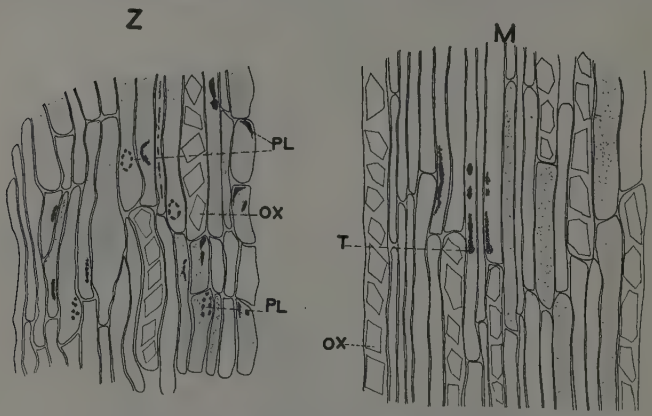


Fig. 5.—Longitudinal section through vascular bundles of zinc-treated (*Z*) and of mottled orange leaves (*M*). (Vital staining with neutral red.) In *Z*, each cell or vessel contains a vacuole (shown by shading) in which the vacuolar solution stains pink with neutral red. Green or yellowish plastids (*PL*) are numerous around the nucleus which, being unstained, is merely outlined by surrounding plastids. In *M*, many of the phloem cells, or vessels, evidence necrosis, for they contain no stainable vacuolar solution, but show (*T*) brown tannic flocculated material; *OX*, calcium oxalate crystals.

vacuoles of the endodermal cells shows a very constant difference in its physical-chemical reaction to the chromates of the killing fluids. In the vicinity of the xylem the vacuolar material is precipitated in the form of globules which stain densely with acid fuchsin. In the vicinity of the phloem the phenolic material seems to have more stability, or perhaps to form more stable complexes in the vacuolar solution; for, when precipitated, it appears as light, fluffy masses filling most of the space within the vacuole.

Calcium oxalate crystals (fig. 4 and plates 1 and 2) occupy the vacuolar cavities of many of the endodermal cells. A longitudinal section shows the abundance and distribution of the crystals (fig. 5). Penzig apparently saw and figured calcium oxalate in the endodermis (Penzig[18], Tav. IV, fig. 1) but made no comments on it. Rufz de Lavison[18] was one of the first investigators to suggest that the endodermis

is the layer which controls the exchange of ions between the vascular system and the surrounding parenchymatous cells. The calcium oxalate crystals in the endodermal cells may result, therefore, from the meeting at that point of the incoming calcium ions with the oxalic acid synthesized in the plant cells.

Spray Treatments.—Additional material for the study of the effects of zinc was obtained from another series of trees which the Division of Orchard Management had sprayed 7 months previously with a solution containing 2.65 per cent commercial zinc sulfate. (The sample used contained 0.001 gram arsenic per pound of $ZnSO_4$, according to analyses kindly furnished by B. M. Laurance. In the concentrations used, the effect of the arsenic is negligible.) The spray was even more effective than the soil treatments previously mentioned. The foliage, which was badly mottled previous to the spray treatment, became green, and the trees, which had made but little growth for several years, produced many new, vigorous shoots. The small, depauperate leaves which were formerly yellow developed new plastids from the inactive mitochondria and showed a healthy green color, although there was no increase in their size. The palisade cells (fig. 2) showed cytological evidence of normal physiological functions. The palisade and adjoining layers of cells contained rather less phenolic material than similar cells from trees to which zinc had been applied through the roots. The older leaves showed a few instances in which the spray material killed cells in the epidermis and palisade layers. Immediately beneath these spots there was a layer of wound tissue. The underlying palisade cells showed evidence of great activity, although the broad shape of the cells was a witness to their former hypoplastic condition.

EFFECTS OF IRON ON THE CYTOLOGY OF LEAVES

Iron salts are known to be essential for green plants, and it is therefore pertinent to inquire whether the lack of green color in mottled orange leaves might be related to a lack of iron. The question has been raised frequently in the past few years on account of the prevalence of chlorosis, mottle-leaf, little-leaf, and other functional disorders of fruit trees. In some instances, there have been marked improvements in the condition of the trees after the application of commercial iron salts to the soil; in other instances no effects were found.

We have studied this question in leaves of orange and pomelo collected from healthy and mottled trees. Significant differences between green and affected leaves with respect to their iron content, however, could not be demonstrated. Sections of orange leaves, both severely

affected and unaffected, were examined by the use of MacCallum's method. The sections were treated first with alcohol containing 3 per cent nitric acid to "unmask" organic iron. After careful washing with neutral alcohol, the slides were placed in the 0.5 per cent hematoxylin for 6 to 18 hours. Then the sections were washed with a mixture of alcohol and ether to remove the uncombined yellow-brown hematoxylin. The inorganic iron forms with hematoxylin, a blue-black substance which can be identified with the microscope. Iron was evident in the remains of plastids and nuclei of affected as well as of healthy leaf tissue. The analysis of the ash of healthy and of mottled orange leaves[10] likewise showed no significant differences in their iron content.

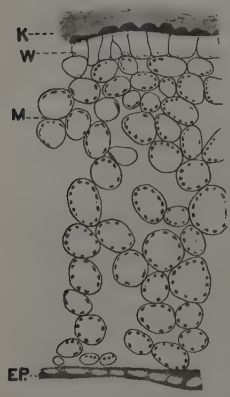


Fig. 6.—Transverse section of orange leaf which had been sprayed with a 2 per cent solution of iron sulfate. *K*, Remains of epidermal and palisade tissues; *W*, wound phellogen; *M*, mesophyll; *EP*, epidermis. (Drawn from section of living leaf, mounted in a 10 per cent cane sugar solution.)

Some material of unusual interest was obtained from an orange tree which had definitely responded to iron sulfate applied as a spray to the foliage. The trees had been heavily sprayed in January with a 2 per cent solution of chemically pure iron sulfate. Many leaves suffered scorch in the vicinity of the midrib where the residual salt was deposited after evaporation of the solution. The leaves showed, however, marked beneficial results from the application of the solution. They formed wound phellogen beneath the necrotic areas and developed a normal green color where previously they had been mottled. The underlying cells subsequently grew and produced a peculiar warping of the leaf. Figure 6 shows the condition of the cells in one of these leaves.

The overlying epidermis and palisade cells had been killed by the iron sulfate, leaving a layer of purplish-brown material beneath which there was a layer of wound tissue (*W*). The mesophyll cells (*M*) showed striking evidence of growth, with a resulting increase both in numbers and in size. The microscopical features thus afforded further evidence of the favorable effect of iron salts on growth. When sections were stained by Mawas' method, it was possible to demonstrate the presence of iron in the nuclei of a large number of cells, especially in those of the wound phellogen. The presence of tannic material in the necrotic layer (*K*) was revealed by its reaction with the iron.

We have obtained rather more precise information about the distribution of iron in the leaf by means of the micro-incineration technic. After having been incinerated (as described in the following section), the slides bearing the mineralized sections were flooded with a 0.5 per cent solution of hematoxylin, washed with water, dehydrated with alcohol, and mounted in balsam. One could see that most of the iron in the palisade cells had been present in the plastids. Confirmatory results were obtained when incinerated sections were treated with a freshly prepared solution containing 0.75 per cent potassium ferrocyanide and 0.25 per cent HCl.

CYTOLOGICAL ANALYSIS BY MEANS OF MICRO-INCINERATION AND MICROCHEMISTRY

Extremely interesting results have been obtained by a method of incineration adapted to the problem under investigation. Policard[14] suggested micro-incineration more than ten years ago as a means for accurate localization of the mineral constituents of the tissues. The success of the method lies in the fact that the incinerated sections (spodograms) preserve in an extraordinary way the topography of the tissues and also of the cells.

We used a mixture of equal parts of 95 per cent alcohol and formalin for killing fluid in order to avoid the introduction of metallic ions which might obscure the true mineral constitution of the tissues. The material was sliced during immersion in the killing fluid to insure good penetration and to avoid premortal translocation of the minerals in the cells. The killed tissue was dehydrated in alcohol, cleared in n-butyl alcohol, embedded in paraffin, and sectioned 6 or 8 microns thick. The paraffin ribbons were fixed to microscope slides with albumen, as for staining with dyes.

The sections were incinerated in an electric furnace where the temperature was gradually raised to 500° C within 3 hours and maintained below 600° C for 2 or 3 hours longer. The furnace was not opened after switching off the current until the slides were cool. Atmospheric dust, as Policard[14] pointed out, is a source of error. We kept the slides under glass covers as much as possible, even in the electric furnace during incineration.

The incinerated sections preserved histological and cytological details of the tissues and adhered to the glass so well that they could be mounted in balsam or treated with various reagents without destroying the patterns. We found it better, in most cases, to study the spodograms in a dry condition without balsam. The area was outlined with four strokes of a wax pencil; this made a ridge thick enough to support the cover

glass and prevent it from coming in contact with the ash. The cover glass was sealed with a marginal seal of hot paraffin.

Since the ash adhered to the slides so well, it was possible to identify zinc and iron and to determine their distribution in the tissues. Comparisons were made with control sections stained by the usual methods.

We have had the best success in identifying zinc *in situ* in the tissues by the use of the Bradley[8] test developed for the identification of zinc in shellfish. It ultilizes sodium nitroprusside in concentrated solution

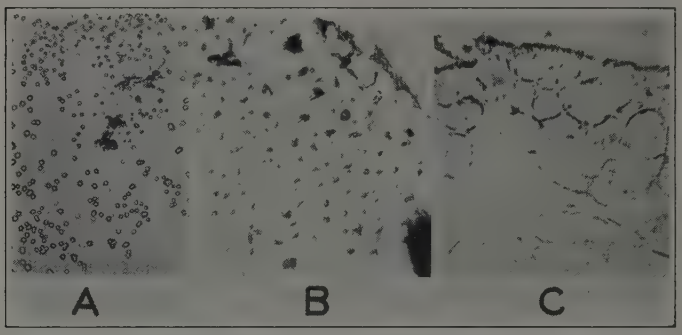


Fig. 7.—*A*, Photomicrograph of zinc nitroprusside formed by adding a drop of concentrated sodium nitroprusside solution to a drop of 1:100,000 solution of zinc chloride. *B*, Photomicrograph of ash of a section of a leaf from a tree to which zinc sulfate had been applied 20 months previously. *C*, Photomicrograph of the ash of a section of a leaf from a tree which had received no zinc sulfate.

which forms, with zinc, $Zn \cdot NO \cdot Fe(CN)_5 \cdot H_2O$, zinc nitroprusside, a salt of low solubility which is precipitated *in situ* on the slide as faintly brownish grains or disks catenulated into botryoidal masses. On standing, these grains may unite to form imperfect cubes, octohedrons, or dodecahedrons, as shown in figure 7*A*, which is a control photomicrograph of a precipitate formed by adding a drop of concentrated sodium nitroprusside solution to a drop of 1:100,000 solution of zinc chloride on a slide, decanting the excess liquid, and focusing on the crystals which had been formed (Chamot and Mason[4]). The crystals of zinc nitroprusside are isotropic. A photomicrograph of crystals obtained by treating the ash of an orange leaf from a tree to which zinc sulfate had been applied 20 months previously shows similar botryoidal masses (fig. 7*B*). A photomicrograph of the ash of a section of a leaf from a tree which had not received zinc sulfate yielded no crystals; the ash merely outlined the pattern of the cell wall (fig. 7*C*).

Manganese forms a nitroprusside which is indistinguishable from that of zinc, and we recognize that some of the crystals found were not

those of zinc. However, the intimate relation between the application of zinc to the soil and the presence of nitroprusside crystals in the ash of incinerated leaf sections makes it improbable that any appreciable amount of the crystalline material observed was manganese nitroprusside.

The presence of zinc in the leaves of treated orange trees has, moreover, been definitely established by analyses of their ash by its highly specific reaction with potassium mercuric thiocyanate. Four samples

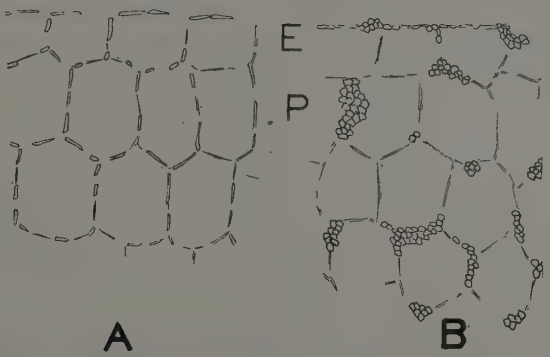


Fig. 8.—Incinerated sections of palisade layers of orange leaves: *A*, from tree which had not received zinc sulfate, shows no crystals of zinc nitroprusside; *B*, from tree having benefited from a soil application of zinc sulfate 20 months previously, shows abundance of crystals of zinc nitroprusside around palisade cells. *E*, Epidermis; *P*, palisade cells.

of orange leaves were collected, dried, and incinerated in crucibles. The samples were:

1. Control: leaves from mottle-leaf trees to which no zinc had been applied.

2. Healthy green leaves which had developed on trees subsequent to the application of zinc sulfate as a spray.

3. Old leaves from same tree as No. 2, marked with small necrotic spots resulting from the zinc sulfate spray applied 7 months previously.

4. Mature leaves from trees sprayed 10 days previously with zinc sulfate plus lime and still carrying the spray residue.

The samples of ash were extracted with dilute sulfuric acid, washed, and filtered. The clear filtrates were then examined for zinc by a modification of the method given by Hammond.[8] A drop of 0.1 per cent $CuSO_4$ solution and a few drops of a dilute solution of freshly prepared potassium mercuric thiocyanate were added to the filtrate. The solution was boiled and cooled.

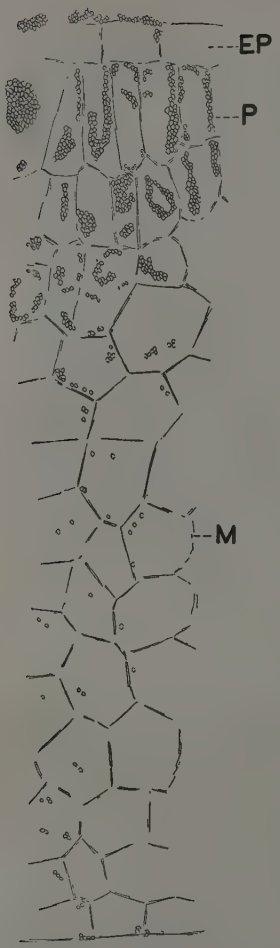


Fig. 9.—Ash of a transverse section of a dwarfed orange leaf which turned green after having been sprayed with zinc sulfate. The treatment with sodium nitroprusside revealed the presence of zinc as crystals of zinc nitroprusside almost exclusively in the palisade cells. *EP*, Epidermis; *P*, palisade cells; *M*, mesophyll.

By this method, violet crystals indicate the presence of zinc. If the amount was small, the violet crystals were not seen readily; therefore a drop of the sediment in the bottom of the test-tube was removed with a pipette and examined under the microscope. The crystals could then be seen readily. The following results were obtained from the samples analyzed:

1. Control: no violet crystals
2. Violet crystals present, but not numerous
3. Violet crystals numerous
4. Violet crystals present in abundance

This is in harmony with the results of the histo-chemical analyses with sodium nitroprusside and confirms the conclusion that the crystals obtained were formed principally by zinc.

The beneficial effects to citrus of an application of zinc sulfate to the soil are therefore concomitant with an absorption of zinc whose distribution in the leaf cells is evident after incineration.

Figure 8 shows camera-lucida drawings of two incinerated sections of orange leaves. Both drawings show the broad, isodiametric palisade cells characteristic of mottled orange leaves. No crystals of zinc nitroprusside occur in 8*A*, the control section, but they are evident in *B*, the ash of the leaf from a tree which received an application of zinc sulfate to the soil 20 months previously. The crystals occur in this material chiefly at the periphery of the palisade cells.

Especially illuminating results were obtained from the study of material from trees which the Division of Orchard Management had sprayed 6 months previously with a 2.65 per cent solution of zinc sul-

fate and which had had an extremely beneficial effect in ameliorating mottle-leaf and promoting growth. Figure 9 shows how the zinc was distributed in the cross section of a leaf. Crystals were found in large numbers in the palisade and intermediate cells, but were scarce in the mesophyll and epidermis cells. It should be stated that the leaves under dis-

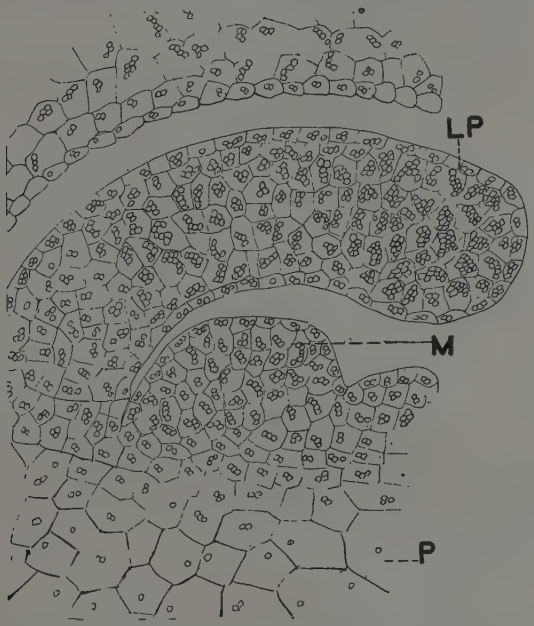


Fig. 10.—Incinerated section of an orange bud from a shoot developed since the tree was sprayed with zinc sulfate. The drawing shows the accumulation of zinc in the leaf primordium and apical bud. *LP*, Leaf primordium; *M*, meristem of bud; *P*, parenchyma.

cussion were formed after the trees had been sprayed. The distribution of the zinc was due, therefore, to physiological processes and not to the deposition of spray material on the foliage. The crystals of zinc nitroprusside were more numerous in the lumina of the cells than in the case previously noted. Mention has already been made of the close connection between chlorophyll-bearing cells and zinc, which has been clearly demonstrated by Bertrand and Andreitcheva.[1]

There is a striking accumulation of zinc in the meristematic tissues of buds of trees to which zinc sulfate was applied, either as a solution on the foliage or to the soil in which the trees grew. Figure 10 shows part

of an incinerated section of a bud from a shoot which developed after the tree had been sprayed with zinc sulfate solution. The incinerated section had been treated with a solution of sodium nitroprusside, as previously described. The crystals of zinc nitroprusside were most numerous in the apical portion of leaf primordia and in the cone of meristem, in contrast to their numbers in the more mature parenchyma cells. The walls of the embryonic cells do not seem to be highly mineralized, and as a consequence were not conspicuous in the spodograms.

Buds collected from shoots of comparable age on unsprayed trees in adjacent rows, incinerated and treated in similar manner, showed no zinc nitroprusside crystals.

DISTRIBUTION OF CALCIUM

The tissues of citrus leaves contain numerous crystals of calcium oxalate, which are generally large in comparison with size of the cells. It is well known that calcium oxalate crystals generally form in vacuoles which are very rich in material that stains deeply with basic dyes. The evidence thus far obtained indicates that this material is composed largely of pentosans. After incineration, these calcium oxalate crystals leave residues which are very conspicuous. A word about calcium seems appropriate at this point, though the significance of the element cannot be fully discussed.

The localization of calcium oxalate can be studied either on freehand sections of living material (fig. 5) or in the ash of micro-incinerated sections of material killed with a mixture of alcohol and formalin.

Calcium oxalate crystals in fresh material or crystals of calcium oxide resulting from their micro-incineration are brightly illuminated by oblique illumination on the dark field. Figure 11, drawn with the help of a $\frac{1}{7}$-inch oil-immersion objective fitted with the diaphragm for dark-field illumination, shows the comparative aspect of ash from the transverse sections of vascular tissues and adjoining parenchyma. In figure 11*M*, from a mottled leaf, there is little calcium in the palisade tissues, as contrasted with the abundance of large crystals in the subepidermal cells of figure 11*Z*, from the leaf of a tree which recovered from its mottled condition after the application of zinc to the soil.

The endodermis around the vascular bundles of the treated leaf is made evident by the abundance of calcium in almost all cells. The endodermis of the mottled leaf can barely be made out, except in the vicinity of the bast fibers, where a few crystals of calcium oxide are seen. The walls of the xylem and of the bast fibers, and even those of the cambium and phloem, are more heavily mineralized in the treated leaf (fig. 11*Z*), where the histological pattern is well preserved, than in the mottled leaf,

where it is barely discernible. The long palisade cells of the treated leaf also contain more ash constituents than the broad and short palisade cells of the mottled leaf.

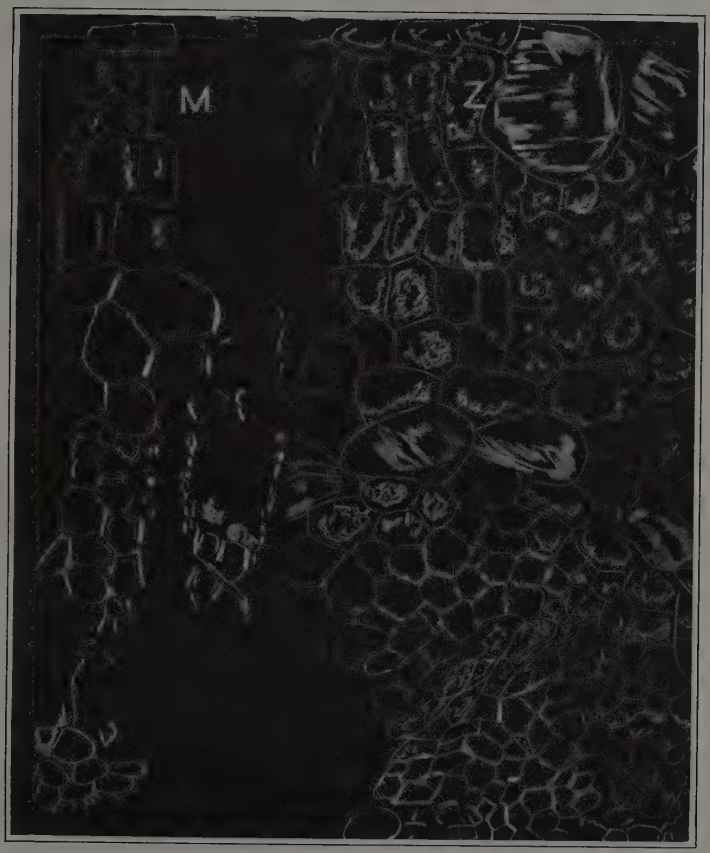


Fig. 11.—Incinerated sections of mottled (*M*) and zinc-treated (*Z*) orange leaves shown by dark-field illumination. Explanation in text.

The incinerated sections of buds also show conspicuous deposits of calcium in cell walls before any solutions are employed for microchemical tests. The walls of the embryonic cells leave a difficultly detectable residue, while those of the older postembryonic cells are clearly outlined by their mineralized residues.

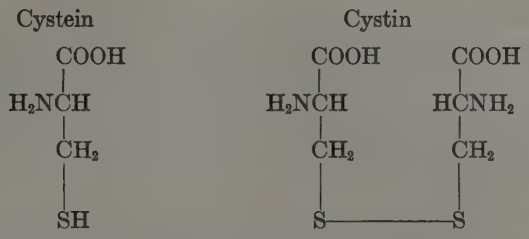
It is known that meristematic cell walls consist of complexes of cellulose, pectin, and fatty acids. Their middle lamellae consist of a protein-pectin complex (Tupper-Carey and Priestley[19]). In the adult parenchyma, the composition of the cell wall is similar to that of the meristem, but the middle lamellae are composed of calcium pectate (Mangin[11]) and calcium soap. The process of micro-incineration clearly reveals the distribution of calcium salts in the tissues. The first evidence of calcium was detected in the primordia of leaves enfolding the apical bud. Calcium oxalate crystals were found there in great abundance in buds from mottled shoots, but were far less abundant in buds on shoots from zinc-treated trees. This apparent contradiction of an excess of calcium in buds of leaf primordia of mottled citrus and deficiency of calcium in adult leaves deserves further consideration. It will be recalled (Reed and Dufrénoy[16]) that starch is also accumulated early in leaf primordia of mottled citrus, while starch synthesis is inhibited in adult leaves. Further investigations will be needed to show the relations of zinc and calcium to organic acids in cellular metabolism.

RELATION OF ZINC AND IRON TO CELL METABOLISM IN MOTTLED LEAVES

Cytological studies of citrus trees affected with mottle-leaf have led us to believe that the pathological symptoms are correlated with an accumulation of suboxidized metabolic substances which result from a low oxidation-reduction potential. The beneficial effects of zinc or iron salts upon mottled citrus leaves are so striking that it seems appropriate to discuss their relation to the sulfhydryl compounds in the cell, and to consider the system whereby the oxidation-reduction potential is determined only by the reduced form.

If the cell is to perform its life processes, it must maintain energy at a given level by oxidation; this level is defined by the oxidation-reduction potential. The ability of the cell to perform the oxidations through which energy is obtained for the maintenance of living processes is largely determined by the oxidation-reduction potential.

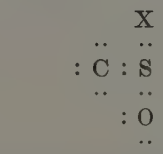
It seems evident, from the work of Hopkins and others, that certain sulfhydryl compounds, such as cystein, are present in all living cells and that they may control processes of oxidation and reduction. It is also known that salts of certain metals catalyze the partial oxidation of cystein to cystin. The oxidation of cystein to cystin seems to be a far more involved process than it was assumed to be when it was represented as the union of two molecules of cystein into one molecule of cystin with the concomitant removal of hydrogen:



The process probably cannot be adequately represented by:

$$2\ RSH \rightleftharpoons RSSR + HH$$

Since sulfur has two unoccupied electron pairs, it can be assumed that an atom of oxygen can be joined to the S atom, with resulting formation of a sulfoxide:



To obtain any appreciable amount of oxidation, it may be necessary for a molecule of sulfoxide to unite with one of sulfhydryl through the formation of complexes with metals which readily change their valences, thereby serving for H and O transfers.

Two or more molecules of amino acids may be linked with one of a metal such as Fe, Cu, Mn, or Zn. The catalytic effect of iron on the rate of oxidation of amino acid has been studied. It was found that there was a proportionality between the rate of oxidation and the percentage of iron present (above a certain minimum). The auto-oxidation activity of the amino acid seems to be related to this proportionality.

The demonstration of the specific effect of zinc in stabilizing the nitroprusside color reaction of glutathione by Giroud and Bulliard[6] confirms our belief that zinc had much to do with the activity of sulfhydryl compounds in regulating the oxidation-reduction potential within the cells of citrus.

This idea seems to be supported by the following observations:

1. The large accumulation of zinc in the meristematic cells of buds and in the palisade cells of leaves.

2. The resumption of activity in the cells of leaves after the trees had been sprayed with zinc sulfate solution, evinced by the normal nuclei, the fibrillar cytoplasm, and the development of normal chloroplasts.

3. The accelerated growth of new shoots on trees subsequent to the application of zinc and the accumulation of zinc in the tissues.

The effects of zinc applications suggest that some reaction has been initiated by which the proteins and carbohydrates of the cells have been utilized to supply energy to the cells, and we have evidence that this is a process of oxidation in which the sulfhydryl compounds play a controlling rôle.

The sterides characteristic of cells of mottled citrus leaves were described in a previous paper (Reed and Dufrénoy[16]). The name "sterinoplast," which had been coined by Guilliermond, was applied to the highly refringent spheres of phytosterol material, or lecithin, found in the epidermal or adjoining palisade layers of leaf cells. The size of the sterinoplasts made it evident that the fatty material in them was not emulsified; and their absence in cells of unmottled leaves lent support to the idea that they were suboxidized products of the metabolism of proteins and carbohydrates. The sterides were comparatively scarce in leaves to which zinc was applied. Apparently, the stabilizing of the sulfhydryl compounds promoted the oxidation of cell metabolites and thereby liberated energy for vital processes.

SUMMARY

The results of these investigations afford evidence that mottle-leaf of citrus is characterized by a shift in the oxidation-reduction equilibrium of the leaf cells. This relation is demonstrated by the results of certain chemical analyses such as those which show that nitrites exist in the expressed sap of mottled leaves, but not in that of green leaves. Indications of a reducing action in the palisade cells of mottled leaves are also shown by their power to reduce methylene blue and Nile blue A.

It is shown in the present investigation that profound changes in the cytological conditions are associated with the recovery of mottled trees after the application of zinc, either through the soil or in the form of a spray on the foliage. In the green leaves of new shoots whose growth had been promoted by zinc applications, neither calcium deficiency nor phloem necrosis is evident, while chloroplastids develop to fair size and form starch. The beneficial effects are especially striking when old, depauperate leaves are sprayed with a solution of zinc sulfate. Although their histological organization is not changed, there is evidence of marked cytological restoration.

The beneficial effects of iron salts on hypoplastic cells of mottled leaves are negligible. Cytological investigations showed that iron can be detected even in the degenerated plastids of hypoplastic cells.

The distribution of iron and zinc in the plant tissues was determined

by a combination of micro-incineration and micro-analysis. It was found that the ashes preserve the histological and cytological features of the tissues sufficiently to afford definite information concerning the distribution of these elements in leaves and buds.

The fact that zinc accumulates in buds which are to produce green tissues and in the palisade cells of green leaves indicates that it is intimately concerned with the oxidation-reduction potential of the cell.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to J. Enderud, who assisted in preparing stained material for the experiments discussed in this paper and in making the illustrations. Acknowledgment is also due Dr. K. V. Thimann, of the California Institute of Technology, who brought to the writers' attention Hammond's method of determining zinc by the use of potassium mercuric thiocyanate.

LITERATURE CITED

[1] Bertrand, G., and M. Andreitcheva.
1933. Sur la teneur comparée en zinc des feuilles vertes et des feuilles étiolées. Compt. Rend. Acad. Sci. [Paris] 197:1374–1376.

[2] Bertrand, G., and B. Benzon.
1929. Sur la teneur en zinc des principaux aliments d'origine végétale. Ann. Inst. Pasteur 43:386–393.

[3] Bradley, H. C.
1906. A delicate color reaction for copper and a microchemical test for zinc. Amer. Jour. Sci. 22:326.

[4] Chamot, E. M., and C. W. Mason.
1931. Handbook of chemical microscopy. 2 vol. xxii + 885 pages. Illus. J. Wiley and Sons, New York.

[5] Chandler, W. H., D. R. Hoagland, and P. L. Hibbard.
1932. Little-leaf or rosette of fruit trees. II. Effect of zinc and other treatments. Amer. Soc. Hort. Sci. Proc. 29:255–263.

[6] Giroud, A., and H. Bulliard.
1933. Réaction des substances à function sulfhydryle. Méthode de mise en evidence dans les tissus. Protoplasma 19:381–384.

[7] Haas, A. R. C.
1932. Some nutritional aspects in mottle-leaf and other physiological diseases of citrus. Hilgardia 6(15):483–559.

[8] Hammond, Wesley H.
1928. A rapid method for the detection of zinc in the presence of iron. Chem.-Anal. 17:14.

[9] Johnston, J. C.
1933. Zinc sulfate, promising new treatment for mottle-leaf. California Citrograph 18:107, 116–118.

[10] Kelley, W. P., and A. B. Cummins.
1920. Composition of normal and mottled citrus leaves. Jour. Agr. Research 20:161–191.

[11] Mangin, L.
1891–1893. Recherches sur la distribution des composés pectiques chez les végétaux. Jour. Botanique 5:400; 6:206, 238; 7:336–337.

[12] Mazé, P.
1914. Recherches de physiologie végétale. IV. Influences respectives des éléments de la solution minérale sur le développement du mais. Ann. Inst. Pasteur 28:21–68.

[13] Penzig, O.
1887. Studi botanici sugli agrumi e sulle piante affini. 590 p. and atlas of 58 pl. Tipographia eredi Botta, Roma.

[14] Policard, A.
1923. Sur un méthode de microincineration applicable aux recherches histochimiques. Bul. Soc. Chim. France 4ème ser. 33:1551.

[15] Reed, H. S., and J. Dufrénoy.
1933. Effets de l'affection dite "mottle-leaf" sur la structure cellulaire des *Citrus*. Revue Gén. Bot. 46:33–44.

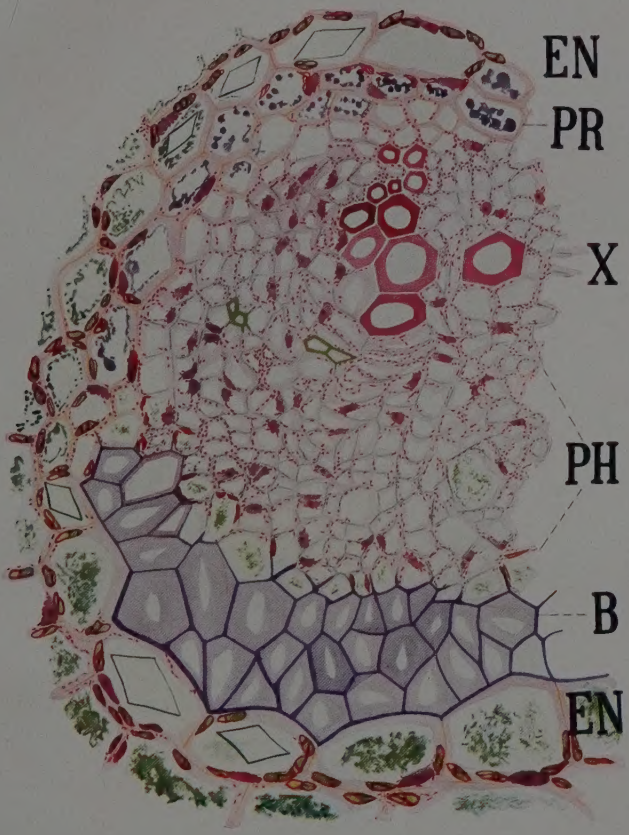
[16] Reed, H. S., and J. Dufrénoy.
1934. Modifications in cell structure accompanying mottle-leaf of the orange. Amer. Jour. Bot. (In press.)

[17] Reed, H. S., and A. R. C. Haas.
1924. Nutrient and toxic effects of certain ions on citrus and walnut trees, with especial reference to the concentration and pH of the medium. California Agr. Exp. Sta. Tech. Paper 17:1–75.

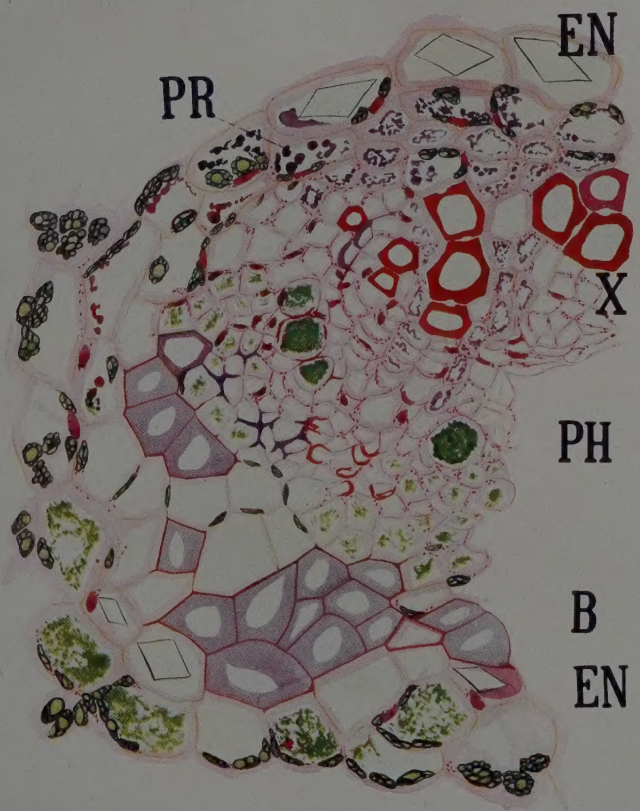
[18] Rufz de Lavison, J.
1911. Recherches sur la pénétration des sels dans le protoplasme et sur la nature de leur action toxique. Ann. Sci. Nat., Bot. 14:97–193.

[19] Tupper-Carey, R. M., and J. H. Priestley.
1923. The composition of the cell-wall at the apical meristem of stem and root. Roy. Soc. [London] Proc. Series B 95:109–131.

[20] Vaile, R. S.
1922. Fertilizer experiments with citrus trees. California Agr. Exp. Sta. Bul. 345:465–512.



Section of a vascular bundle in a healthy orange leaf. *EN*, Endodermis; *PR*, pericycle; *X*, xylem; *PH*, phloem; *B*, bast.



Section of a vascular bundle in a mottled orange leaf for comparison with plate 1. *EN*, Endodermis; *PR*, pericycle; *X*, xylem; *PH*, phloem; *B*, bast.